JOURNAL OF AGRICULTURAL RESEARCH

VOL. 67 WASHINGTON, D. C., NOVEMBER 15, 1943 No. 10

MEASUREMENT OF THE RESISTANCE OF PEAS TO APHIDS[1]

By C. D. HARRINGTON, formerly *industrial fellow in economic entomology and genetics*, ED. M. SEARLES, *assistant professor of economic entomology*, R. A. BRINK, *professor of genetics*, and C. EISENHART, *station statistician, Wisconsin Agricultural Experiment Station*.[2]

INTRODUCTION

Studies looking toward control of the pea aphid, *Macrosiphum* (*Illinoia*) *pisi* (Kalt.), through use of aphid-resistant pea varieties were begun at Wisconsin in 1930 (*7*).[3] Determination of the aphid population at successive intervals during the season on peas growing in the field showed that varieties differ in the number of aphids borne upon them, and that certain varieties are relatively resistant (*8*). These early findings were corroborated by Maltais in 1936 (*5*). Initial attempts to approach the problem from the breeding standpoint were abandoned, however, because the procedure then in use was limited to testing plants en masse and could not be adapted satisfactorily to the detection of resistance in small segregating populations or in individual plants. In addition, data secured by means of the field procedure were not always amenable to satisfactory statistical analysis because of the multiplicity of factors affecting aphid populations under field conditions. In many cases, aphid populations were decimated by storms or by aphid parasites, predators, and disease before sufficient data could be obtained to show the comparative resistance of peas under test. Because of these disadvantages, it became apparent that before much progress could be made in breeding for aphid resistance in peas, a more dependable testing procedure would have to be developed. It was recognized that the new procedure must be able to give a rapid test with small numbers of plants, and that the data must lend themselves to satisfactory statistical analysis.

In 1939 major emphasis was shifted from field to greenhouse investigations, where detailed studies of host-parasite relationships pointed the way toward development of an accurate testing procedure (*4*). The new procedure is primarily adapted for greenhouse use, but may be used, with modified equipment, under field conditions as well. The principal advantages of the new procedure are: (1) Rapidity, (2) accuracy under variable environal conditions, and (3) adaptibility to the requirements of the pea breeder. The new procedure for measuring aphid resistance is described in this paper and an account is given of some results obtained with it. Considerable attention is given to the statistical analysis of the data because of the introduction of special statistical procedures.

[1] Received for publication September 22, 1942. Paper from the Departments of Economic Entomology and Genetics (No. 294), Wisconsin Agricultural Experiment Station. The work has been supported in part by the Associated Seed Growers, Inc., of New Haven, Conn.

[2] The writers acknowledge their indebtedness to J. E. Dudley and T. Bronson, of the U. S. Department of Agriculture, and to H. F. Wilson and C. E. Dieter, of the Department of Economic Entomology of this station, for helpful advice in the course of the investigation, and to Frieda S. Swed and Mary Ann Lee, of the Agricultural Statistical Service, for calculations and other assistance in connection with tables 2, 3, 4, and 8.

[3] Italic numbers in parentheses refer to Literature Cited, p. 387.

BIOLOGICAL BASIS OF THE EXPERIMENTAL PROCEDURE

The term "resistance" in this paper denotes the inherent ability of the plant to ward off or resist insect attack through an inhibitive influence on the insect biology. Susceptibility is considered the opposite of resistance. Both terms, however, are relative, there being no clear-cut line between plants which are slightly resistant and those which are susceptible; i. e., plants in which no inhibitive influence on the insect biology can be detected. This paper is not concerned with "tolerance," which may be described as the ability of a plant to support its insect population. The fact that a vigorous plant growing under ideal conditions can support a larger insect population than a weak plant in a poor environment exemplifies tolerance. Such plants may be equally resistant or susceptible.

Growth of the pea plant involves a progressive unfolding and expansion of parts arising from the meristematic tissues of the apical bud. This growth habit results in a gradient within the plant with respect to age of tissues, the apical tissues being youngest, those of the basal parts oldest. Within individual plants, greenhouse studies [4] have indicated that the rate of aphid development and reproduction, and the length of life of the insect, are inversely proportional to the age of tissues upon which the insects are confined. The inhibiting influence of older plant tissues appears to be associated directly with added age, and is independent of the variety of peas upon which it occurs. Differences in resistance between plants can be detected by differentials in developmental rates, reproductive rates, or in longevity of aphids confined upon any region of the test plants, provided that age of tissues used is identical for all plants under test (*4*).[4] If the aphids are confined to apical plant tissues, differences in total plant age may be overlooked, provided the test is run before blossoming and fruiting occur. Physiological changes of an unknown character associated with reproduction in the plant tend to cause irregular variations in aphid behavior.

The feeding of the pea aphid is not characterized by acute stigmonose, the injury apparently being confined to the withdrawal of necessary plant juices. Individual aphids cause, therefore, but slight damage, severe injury appearing only after sustained feeding by relatively large numbers of the pest. Because of this host-parasite relationship, the ultimate amount of injury to pea plants is determined principally by the numbers of aphids present, the duration of the infestation, and the tolerance of the host, i. e., its ability to replenish lost plant juices. The superiority of resistant over susceptible plants is due principally to the fact that their aphid populations increase at a slower rate, the insects requiring a longer infestation period to reach damaging numbers. The principal factor retarding population increases on resistant plants is the inhibition of aphid reproduction. Because of this fact, the criterion of resistance used in this technique is the rate of aphid reproduction on the plant under test.

Since temperature influences aphid reproduction, it must be carefully considered in utilizing data on aphid reproductive rates. In general, the velocity of aphid reproduction increases proportionately

[4] HARRINGTON, C. D. Unpublished data.

with rise in temperature, beginning at the developmental threshold (about 43° F.) and reaching a sustained maximum at 70° to 75° F. At higher temperatures, reproduction may be even faster for a time, but is not maintained continuously at these levels. Daily fluctuations of greenhouse temperatures cause comparable fluctuations in daily numbers of progeny produced by aphids, even though the insects are confined upon plants of similar resistance or susceptibility. When aphids are confined upon plants that differ in resistance, the daily number of progeny produced upon each will vary with day-to-day temperatures, but proportionately fewer young will always be produced on the more resistant plants.

If a number of plants of varying resistance are individually infested with a single newly reproducing aphid from a uniform stock, progeny counts after 6 to 8 days will reveal the comparative resistance of the plants tested. The value secured for each plant, however, cannot be safely compared with values secured for plants tested in previous or subsequent trials unless allowances can be made for temperature differences. In a comprehensive testing program the number of plants to be evaluated may be too large to include in a single trial. The first method used of terminating an experiment after a given interval of time, regardless of temperature, was abandoned, therefore, in favor of a system in which temperature is also a factor in determining the duration of the trial. This led to the adoption of accumulated "degree-hours" as the basis for deciding when a given experiment was to be concluded. According to this procedure, a trial is terminated, not after a given interval of time has passed following the initial infestation, but when a given number of effective degree-hours have accumulated. With this system there is nearly an automatic compensation in time for trials run under fluctuating temperatures. When prevailing temperatures approach the developmental threshold, effective degree-hours accumulate more and more slowly, thus prolonging the trial period. As the temperatures rise, the trial period is proportionately shortened. Merriman's developmental threshold of 43° F. is used as the base line, temperatures above this point being considered as effective in promoting aphid reproduction.

Within the optimum testing range of 55° to 75° F., changes in velocity of aphid reproduction are closely proportional to changes in rate of accumulation of effective degree-hours. Because of this relationship the number of aphids produced upon plants of similar resistance would be expected to vary only slightly from trial to trial. In greenhouses not equipped with devices for automatic control of temperature, however, temperatures often fluctuate beyond the optimum testing range. When fluctuations below 55° occur, aphid reproduction takes place at a slightly greater rate than effective degree-hours accumulate, while at temperatures above 75° the opposite is true because of an inhibiting influence of high temperatures on aphid reproduction. When temperatures fluctuate outside the optimum testing range, the average temperature of the experiment calculated at its completion is useful in indicating the extent to which the rate of aphid reproduction has deviated from the rate of accumulation of effective degree-hours. The importance and use of average temperature values will be discussed in a later section.

Because of the nearly straight-line relationship between rates of aphid reproduction and rates of accumulation of effective degree-hours, it would be expected that in a given number of accumulated degree-hours newly reproducing aphids of uniform stock confined upon comparable tissues of plants varying in resistance would repeatedly produce numbers of progeny in direct proportion to the congeniality of the host plants. This is the biological basis of the new testing procedure.

MATERIALS

Thirty varieties of field, garden, and canning peas (*Pisum sativum*) and one variety of English broadbean (*Vicia faba*) were used during the development and standardization of the techinque. Of the pea varieties, Perfection, Alaska, Gradawax, Onward, Pride, and Wisconsin Penin were most frequently employed. The plants were grown in soil in 4-inch pots or in No. 2 enameled tin cans with the sand culture method.

The aphid stock originated from a single agamic apterous insect collected from alfalfa. Since the pea aphid reproduces parthenogenetically, the desendants of this individual are presumably genetically identical. The use of pure-line insects was adopted to avoid differences in vigor which might occur in a random aphid population because of possible genetic heterogeneity. The aphid stock was maintained on the susceptible Perfection variety, growing in 6-inch pots in soil, and covered with cylindrical screen cages (fig.1).

FIGURE 1.—Pots of Perfection peas planted at intervals to insure a continual supply of succulent plants for the aphid stock which is confined in cages at the rear. Note the sand layer over the soil to insure a tight fit between cage and pot

Mechanical equipment consisted of gauze-covered, clear-glass lantern globes for confining the aphids to the trial plants, and a series of racks for support of the plants and lantern globes. Ventilation was accomplished by directing the air stream from a number of 8-inch electric fans over the globes. Aphid progenies were removed from the plants with a mechanically powered aspirator constructed from a small electric vacuum cleaner. Temperature fluctuations during the progress of the trials were recorded by a thermograph. The arrangement of the equipment is shown in figure 2.

FIGURE 2.—Arrangement of testing equipment showing method of mounting plants in the testing racks, and the power-driven aspirator used to remove the aphid progenies from the plants at the close of the test. Note the detachable vial of the aspirator, and the filing system used to store the progenies until the counts could be made.

THE TESTING PROCEDURE

Plants to be tested are planted in individual containers and grown under as uniform conditions as possible. When the plants reach 4 to 5 inches in height (18 to 20 days after planting) the containers are mounted in the racks as shown in figure 2. A single freshly reproducing agamic apterous aphid is placed upon the tip of each

plant with a camel's-hair brush, after which a lantern globe is set over the plant. The racks are placed in random order on a bench, the time is recorded, and the electric fans adjusted to insure ventilation of the globes (fig. 3).

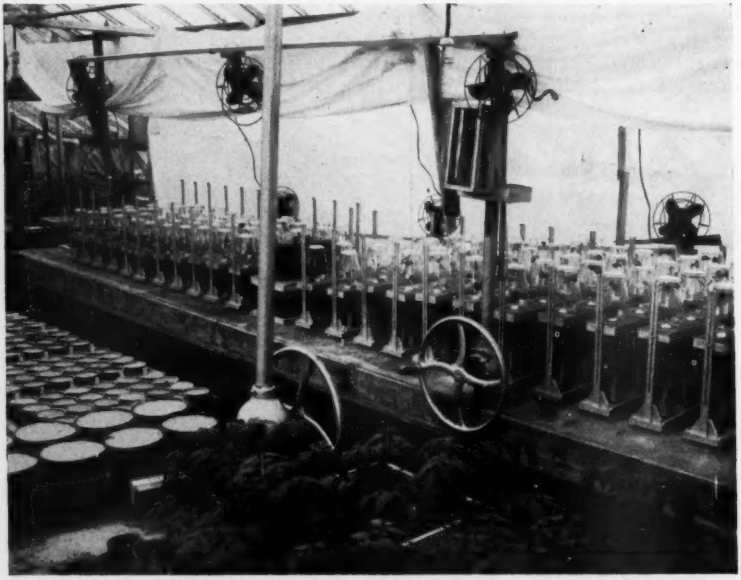

FIGURE 3.—Experimental set-up showing 22 testing racks used in a varietal test. Note the position of the thermograph and of the ventilation fans in the background. The fan rack in the foreground was raised in order to obtain a clearer view.

The aphids used in infesting the test plants are obtained from stock pots infested 8 to 9 days previously with 20 to 25 reproducing aphids. The parent aphids are allowed to reproduce on the plants only 4 hours, after which they are removed. The progeny produced during this short period are relatively unform in age and vigor, and are of like genetic constitution. These aphids are placed on the test plants upon reaching reproductive maturity. It should be pointed out, however, that the stock plants must be in good condition and not overcrowded if a uniformly vigorous stock of apterous aphids is to be obtained. The presence of alate aphids in the aphid stock indicates that the insects may have suffered from lack of sufficient food.

The trial is ended when 4,750 effective degree-hours have accumulated. This number was found to be most satisfactory because it permits the parent aphids to produce the greatest possible number of progeny, yet stops the trial before their first-born young begin to reproduce. The results thus represent the reproductive efforts of the parent aphids alone. At average temperatures of 68° to 70° F., this number is reached on the eighth day.

At completion of the trial the aphids are removed from the plants with the power aspirator (fig. 2). The aphids on each plant are drawn into an individual vial which is then placed in a small vial rack numbered to correspond to the larger rack holding the test plant. After all progenies have been removed, the insects are counted. A few drops of chloroform, placed on a cotton plug and moved progressively from vial to vial before counting, anesthetizes the insects satisfactorily.

In addition to the number of progeny, other pertinent data are recorded. The condition of the parent aphid, whether normal, emaciated, diseased, parasitized, or dead, is noted. Normal parent aphids are usually found associated with susceptible host plants, while emaciated aphids, living or dead are usually associated with resistant plants. Low progeny counts resulting from disease or parasitism of parent aphids cannot be differentiated from low counts due to resistance without examination of the parent aphids. Disease or parasitism is rare, however, in adequately managed stocks. The number of progeny reaching the fifth instar before conclusion of the trial is also recorded. The presence of more than 10 of these insects per plant is a warning that second-generation offspring may have been produced. Such an occurrence is unlikely, however, unless the trial period is unduly prolonged.

The testing procedure will give fairly consistent results under widely fluctuating greenhouse temperatures (45° to 90° F.) provided, however, that the average temperature lies between 60° and 75° F. Average temperatures below 60° tend to inhibit reproduction on all plants to such an extent that small differences in resistance are masked. Average temperatures above 75° result in undue magnification of differences in resistance because of increased mortality of parent aphids on the more resistant plants. Although satisfactory results may be obtained between mean temperatures of 60° to 75°, 65° to 70° is the preferable range.

PRELIMINARY TRIALS OF APHID RESISTANCE IN PEAS

Three preliminary trials were made in consecutive order to determine the comparative resistance of six pea varieties—Perfection, Onward, Alaksa, Wisconsin Penin, Gradawax, and Pride. No attempt was made to stop these trials at the accumulation of 4,750 degree-hours, since this number had not been definitely fixed at that time. Trial 1 ran 4,390 accumulated degree-hours at an average temperature of 68.5° F.; trial 2, 5,008 at 68.8°; and trial 3, 4,186 at 67.3°. The results are presented in table 1.

The data in table 1 indicate that the six pea varieties vary considerably in congeniality to the pea aphid. These differences are expressed in each trial by variations in (1) mortality of parent aphids, (2) mortality of progeny, (3) numbers of progeny reaching fifth instar, and (4) average total numbers of progeny produced. It would appear that Perfection is most susceptible and that Alaska is more resistant than Perfection but less resistant than the other four varieties. Increased numbers of progeny on Perfection, Alaska, and Wisconsin Penin in trial 2 as compared with trials 1 and 3, together with increased numbers of progeny reaching the fifth instar, indicate that second-generation reproduction occurred during the additional time this trial was permitted to run.

TABLE 1.—*Aphid reaction on 6 pea varieties*

Test No.	Variety	Average parent mortality	Average progeny mortality	Average number of fifth instars	Average total progeny
1	Perfection	0	0	9.4	68.6
	Alaska	1	.6	8.4	50.7
	Wisconsin Penin	3	1.0	5.3	33.1
	Gradawax	7	2.5	4.0	29.4
	Pride	7	1.3	1.5	27.6
	Onward	6	1.2	1.3	24.2
2	Perfection	0	0	16.5	[1] 91.5
	Alaska	1	0	16.3	[1] 77.3
	Wisconsin Penin	6	.3	11.0	[1] 45.2
	Gradawax	6	.8	5.3	40.8
	Pride	8	1.3	7.4	33.7
	Onward	6	1.2	9.8	35.6
3	Perfection	0	----------	7.0	61.3
	Alaska	2	----------	6.6	50.7
	Wisconsin Penin [2]	7	----------	5.3	37.9
	Gradawax	6	----------	3.2	38.4
	Pride	4	----------	3.3	39.3
	Onward	9	----------	2.9	36.9

[1] Second-generation reproduction noted.
[2] Counts based on 9 plants, all others on 10 plants.

STATISTICAL TREATMENT OF THE DATA

Since the mathematical theory behind the usual analysis of variance tests of significance presupposes the existence of a common variance appropriate to all observations involved in the analysis, it was deemed well to proceed by first examining within each of the separate varietal trials the tenability of the assumption of a common within-variety variance. For this purpose, the Bartlett homogeneity test (*1*) was used with the results shown in table 2.

TABLE 2.—χ^2 *test of homogeneity of within-variety variances within trials*

Test No.	Degrees of freedom	χ^2
1	5	7.950
2	5	7.548
3	5	9.724
Total	15	25.222

Since the 5-percent significance level of χ^2 for 5 degrees of freedom is 11.07, no one of the observed χ^2's considered alone is statistically significant at this level. On the other hand, their sum, 25.222, is borderline—the 5-percent level for 15 degrees of freedom is 24.996—and throws suspicion on the assumption of a common within-variety variance. An examination of the variance estimates themselves did not reveal any variety or group of varieties which differed *consistently* in this respect from the others. Consequently, it was felt that the usual analysis of variance procedure could be applied without appreciable error to the data of a single varietal test.

Since a composite analysis of variance with data from all three varietal trials would require homogeneity of within-variety variance, not only within trials but also over all three trials, it was decided to find whether the three estimates of within-variety variance for each variety were homogenous. The results are presented in table 3.

TABLE 3.—χ^2 *test of homogeneity of within-variety variances for each variety*

Variety	Over-all trials		Trial 2 versus trials 1 and 3		Trial 1 versus trial 3	
	Degrees of freedom	χ^2 [1]	Degrees of freedom	χ^2 [1]	Degrees of freedom	χ^2 [1]
Perfection	2	8.166*	1	5.750*	1	2.402
Alaska	2	6.544*	1	6.007*	1	.533
Gradawax	2	.673	1	.371	1	.300
Pride	2	.263	1	.000	1	.261
Onward	2	5.025	1	.002	1	4.994*
Wisconsin Penin	2	9.506*	1	7.396*	1	2.097

*The values shown are the adjusted χ^2 values, the adjustment factor not being the same in all 3 columns.
[1] Significant at the 5-percent level.

From table 3 it is evident that the within-variety variances for Perfection, Alaska, and Wisconsin Penin are not homogeneous over the three trials, and that it is the value for trial 2 which is out of line in each case. The analysis has here detected the distortion caused by second-generation reproduction on these varieties in trial 2. If trial 2 data are omitted from the analysis, it appears that the assumption of homogeneity of trials 1 and 3 of the within-variety variance will be acceptable in all cases, except possibly in the case of Onward where early death of parent aphids unduly increased its internal variability.

With these points in mind, a composite analysis of variance was performed, using the data of trials 1 and 3. The results are presented in table 4.

TABLE 4.—*Analysis of variance, data of trials 1 and 3*

Variation	Degrees of freedom	Sum of squares	Mean square
Between varieties:			
Alaska and Perfection vs. others	1	15,983.50	15,983.50*
Alaska vs. Perfection	1	2,030.63	2,030.63*
Among others	3	240.18	80.06
Total	5	18,254.31	3,650.86*
Trial 1 vs. trial 3	1	820.90	820.90*
Varieties × trials:			
Alaska and Perfection vs. others	1	1,047.88	1,047.88*
Alaska vs. Perfection	1	199.83	199.83*
Among others	3	202.36	67.45
Total	5	1,450.07	290.01*
Within varieties	107	12,140.30	113.46
Grand total	118	32,665.58	

*Significant at the 5 percent level when the pooled within-varieties mean square, 113.46, is used to represent the experimental error. This composite mean square includes a portion from the Onward variety which appears to be a bit discordant. However, the exclusion from the analysis of this variety and its contribution to the estimate of "error" only changes the "error" to 114.82, which is a negligible change so far as the present analysis of variance is concerned. Therefore, for completeness, the Onward variety and its contribution to the estimate of "error" were retained.

It will be noted that the 5 degrees of freedom for "Between varieties" and for "Varieties × trials," have been resolved further into the comparison (a) of Alaska and Perfection with the other four varieties considered as a group, (b) of Alaska with Perfection, and (c) of the

other four among themselves. This further decomposition leads to the following inferences on the basis of trials 1 and 3: (1) There are significant differences (at the 5-percent level) between varieties with regard to average aphid production; (2) the varieties Gradawax, Onward, Pride, and Wisconsin Penin do not differ significantly from each other in respect to average number of aphids produced, but do differ significantly from Perfection and Alaska, which in turn differ significantly in this regard. Trial 3 has the higher production.

VARIETAL TRIALS OF APHID RESISTANCE IN PEAS

The data in table 5 were secured from several similar trials of varietal resistance. Values given each variety represent the average number of young produced by 10 aphids confined individually upon 10 plants under the temperature-time conditions of its respective trial.

TABLE 5.—*Comparative aphid resistance of a number of pea varieties as determined by the use of the new testing procedure in 5 separate trials*

Varietal trial No.	Average temperature	Accumulated degree-hours	Variety	Average aphids
	° *F.*			*Number*
1	68.0	4,832	Triumph	88.1
			Early Wales	78.9
			Prince of Wales	77.8
			Profusion	76.3
			Asgrow No. 40	75.7
			Perfection (check)	72.8
			Asgrow No. 83	67.6
			Wisconsin Merit	65.5
			Surprise	62.5
			Yellow Admiral	55.7
2	71.1	4,793	Danby Stratagem	71.1
			Perfection (check)	68.7
			Dwarf Defiance	63.9
			Sherwood	60.2
			Potlatch	54.1
			Stratagem (regular)	44.0
3	72.2	4,675	Perfection (check)	73.1
			Horal	70.4
			Canner King	65.2
			Horsford	62.4
4	70.8	4,759	Fasciated Sweet	77.6
			Perfection (check)	72.7
			Emerald	62.9
			Confidence (dark)	60.3
			Glacier	59.3
			Confidence (light)	57.6
5	70.7	4,714	Perfection (check)	73.2
			Daniel	72.1
			Creole	68.4
			Cade	66.4
			English boardbean [1]	63.9

[1] *Viciafaba.*

THE COMPARISON EQUATION

Reference to the values obtained for the Perfection variety (which was the susceptible check) in the trials listed in table 5 shows that the values vary from trial to trial, although the number of accumulated degree-hours is approximately equal in all. This is because the trials were made under temperatures which frequently fluctuated above the upper limit of the optimum testing range, 55° to 75° F. When such fluctuations occur, the parallel relationship between rate of aphid reproduction and rate of accumulation of effective degree-hours is temporarily lost. Differences in the number and size of these fluc-

tuations between trials caused the differences noted in the values obtained for the Perfection check. From this it is seen that the accumulation of a given number of effective degree-hours does not wholly determine the value obtained when a variety is tested under widely variable temperature conditions. The value is influenced to some extent also by the particular combinations of temperature and time which occur during the trial period. For example, a temperature of 70° F. acting for 4 hours will result in slightly more young aphids being produced than will temperatures of 50° and 73° F. acting for 6 and 2 hours respectively, even though the total number of accumulated degree-hours (108) is equal in both cases. The average temperature in the first instance is 70° while in the latter it is only 56.5°. The key to differences in number of aphids produced is obviously the average temperature. The total number of accumulated degree-hours is actually the sum of all temperature-time combinations occurring in a given period. The average temperature, on the other hand, is an expression of their average magnitude. The interrelation of average temperature and number of accumulated degree-hours is complex with respect to its influence on aphid reproduction. When a given variety is tested a number of times under varying conditions, it is found that the progenies produced in each trial are directly proportional to the products of their respective average temperatures and total number of accumulated degree-hours. This statement may be expressed algebraically by the following equation, hereafter called the "comparison equation":

$$\frac{V_1}{(ADH_1)\ (AT_1)} = \frac{V_2}{(ADH_2)\ (AT_2)}$$

where ADH_1 and ADH_2 denote the accumulated degree-hours and AT_1 and AT_2 denote the average temperatures for the first and second test, respectively, and V_1 and V_2 denote the corresponding expected numbers of progeny.[5] If this equation is valid, a ratio of approximately 1:1 should result when actual values are substituted and the equation simplified. Table 6, based on the data from trials 1 and 3 of the preliminary study and from the five trials of varieties, provides a series of empirical tests of the validity of the comparison equation. The values for V used in the equations are the values obtained for Perfection in the respective trials.

The terms of the ratios presented in table 6 are found to vary less than 5 percent in 18 of the 21 cases, the maximum difference found being 7.8 percent. This indicates that the reaction of the aphids to the Perfection variety is essentially the same when variations in time and temperature are thus taken into account.

The second-generation reproduction which occurred in trial 2 of the preliminary series prevented true estimates of resistance from being obtained for Perfection, Alaska, and Wisconsin Penin in this trial. If the value obtained for Perfection in this experiment is substituted in one side of the equation, table 7, the terms of the resulting ratios are found to vary considerably.

[5] For a given set of time-temperature conditions there will correspond an expected number of progeny about which observed numbers of progeny will vary in successive trials as a result of sampling fluctuations.

TABLE 6.—*Values gained for Perfection under the differing conditions of 7 separate trials substituted in the comparison equation in all possible combinations to test the validity of the equation*

Combination No.	Perfection values, according to trial No., used in equation on—				Ratio
	Left side		Right side		
	Preliminary trial No.	Varietal trial No.	Preliminary trial No.	Varietal trial No.	
1	1	3	3		1.000=1.047
2	1	1		1	1.000=1.000
3	1	2		2	1.000=1.078
4		3		3	1.000=1.053
5		4		4	1.000=1.057
6		5		5	1.000=1.038
7	3			1	1.000=1.048
8	3			2	1.000=1.028
9	3			3	1.000=1.005
10	3			4	1.000=1.008
11	3			5	1.000=1.003
12		1		2	1.000=1.077
13		1		3	1.000=1.052
14		1		4	1.000=1.056
15		1		5	1.000=1.035
16		2		3	1.000=1.023
17		2		4	1.000=1.019
18		2		5	1.000=1.035
19		3		4	1.000=1.004
20		3		5	1.000=1.014
21		4		5	1.000=1.017

TABLE 7.—*Increased variation of the expected 1:1 ratio resulting when abnormal values due to second-geneartion reproduction are substituted in one side of the comparison equation*

Combination No.	Perfection values, according to trial No., substituted in equation on—			Ratio
	Left side	Right side		
		Preliminary trial No.	Varietal trial No.	
1	Preliminary trial No. 2	1		1.000=1.164
2		3		1.000=1.220
3			1	1.000=1.164
4			2	1.000=1.225
5			3	1.000=1.226
6			4	1.000=1.230
7			5	1.000=1.224

It is seen from table 7 that the differences between the two terms of the ratios vary from 16.4 to 23 percent. These computations show that the comparison equation may be used as a means of determining the comparability of data from separate trials. The more closely the resultant ratio approaches 1:1, the greater the degree of comparability between the trials. For practical purposes, two trials may be considered as comparable if the two terms of the ratio do not vary more than 10 percent (1.00=1.10), and the standard errors of the average values of V employed are not greater than 4 percent of these average values. If the standard errors are greater than this, averages based on larger numbers of observations should be employed.

COMBINING RESULTS OF SEPARATE TRIALS

After a number of varietal trials are completed, it may be desired to combine the results so as to show the relative resistance of all varieties tested on a single scale. Three methods of varying accuracy have been devised for this purpose. All are based, directly or indirectly on the comparison equation, and involve formulae whereby values obtained for the different varieties under the conditions of their respective trials are used to estimate the values which might be expected of these varieties under a common set of experimental conditions.

Method No. 1, Use of Perfection Checks

Within a given trial, all varieties are exposed to the same combinations of time and temperature, and from this the assumption can be made that any time-temperature variation which affects reproduction on the Perfection check variety will affect reproduction on other varieties to a proportionate degree. If the reaction of the aphids to the Perfection checks is found to be similar in a series of trials when variations of time and temperature are accounted for by the use of the comparison equation, then the values gained for Perfection can be used as a basis for determining the approximate resistance of all other varieties tested. For example, one may wish to know how Wisconsin Merit, tested in varietal trial 1, compares in aphid resistance to varieties tested in varietal trial 2. The following relation is used, PV_1 and PV_2 here denoting the average number of progeny on Perfection in the respective trials, MV_1 the average number of progeny on Wisconsin Merit in the first trial, and x standing for the estimate of MV_2, the number of progeny expected on Wisconsin Merit under the conditions of the second trial:

$$PV_1 : PV_2 = MV_1 : x$$

$$74.9 : 72.1 = 65.5 : x$$

$$74.9x = 47{,}226$$

$$x = 63.0$$

From this it is seen that Wisconsin Merit, which tested 65.5 in varietal trial 1, would be expected to test 63.0 under the conditions of varietal trial 2. This procedure, when applied to each variety of varietal trial 1, will result in a composite list showing the comparative resistance of all varieties included in the two trials. If results of a large number of trials are to be combined, estimated values of the different varieties may be computed by the above procedure for a set of experimental conditions not represented by any of the trials, provided, of course, that an estimated value of the Perfection check obtained by direct observation is known for this set of conditions.

Method 2, Direct Use of the Comparison Equation

If an estimated value of a variety obtained by direct observation is obtained for a particular set of conditions, use of the comparison equation will show the response which may be expected of this variety

under any other set of experimental conditions. The formula used is as follows:

$$\text{Estimated } V_2 = (\text{observed } V_1) \cdot \frac{(ADH_2)(AT_2)}{(ADH_1)(AT_1)}$$

Using the Wisconsin Merit value observed in variety trial 1 to calculate its response under the conditions of variety trial 2, the following result is obtained:

$$\text{Estimated } V_2 = 65.5 \frac{(4{,}793)(71.1)}{(4{,}832)(68.0)}$$

$$\text{Estimated } V_2 = \frac{22321221}{328{,}576} = 67.9$$

This procedure is more accurate than method 1 since it is based directly on the comparison equation, and does not involve the use of another variety. The reason for this will be discussed later.

Method 3, Estimation by Interval

While an estimate of V_2 obtained by either of the above methods will generally be near the true value V_2, such an estimate gives no indication of its accuracy, and it is highly improbable that it will actually equal the true value V_2. In order to obtain an estimate with which there can be associated a chosen level of confidence, estimation by interval is necessary.[6] Thus, if an interval with a confidence coefficient of 0.95 is employed, the probability is 0.95 that the interval will include the true value within its range. Such an interval for log V_2 is given by

$$\text{estimated average log } V_2 \pm t.05.s$$

where

$$\text{estimated average log } V_2 = \log (ADH_2) + \log (AT_2) - \log (ADH_1) + \log (AT_1) + \text{observed average log } V_1$$

$t.05$ is the 0.05 significance level of Student's t for $N-1$ degrees of freedom (*3*), and

$$s^2 = \frac{\sum (\log V_1)^2 - \frac{(\sum \log V_1)^2}{N_1}}{N_1(N_1-1)}$$

in which $\sum$ denotes summation over the N_1 values of log V_1 supplied by the first test. By taking antilogarithms the corresponding 0.95 confidence interval for V_2 is obtained.

As an example, the data for Perfection of trial 1 is used to predict

[6] Neyman, J. Lectures and conferences on mathematical statistics. 160 pp., illus. [1938.] (U. S. Dept. Agr. Graduate School processed publication.) Washington, D. C.

the interval within which Perfection must lie for the standard conditions (ADH=4,750; AT=68.0° F.):

Perfection V_1 values	Log V_1 values
75	1.875
68	1.832
65	1.813
62	1.792
65	1.813
61	1.785
78	1.892
68	1.832
76	1.881
68	1.832

ADH_1=4,390

AT_1=68.5

ADH_2=4,750

AT_2=68.0

Estimated average log V_2=3.677 +1.832−3,642−1.836+1.835= 1.866 Antilog 1.866=73.5

log V_1=18.347

(log V_1)^2=33.673549

$$s^2=\frac{33.673549-33.661241}{(9)\ (10)}=.00013675$$

s=.0117 $t.05$=2.262 for 9 degrees of freedom

Interval=1.866±0.026

log V_2 ranges from 1.840 to 1.892

V_2 ranges from 69.2 to 78.0

Using method 3 in this case, an average value of 73.5 is predicted for V_2 and the probability is 19 in 20 that the true V_2 lies within the interval 69.2 to 78.0.[7] The V_2 value when predicted by the use of method 2 fell at 73.9 while the average V_2 value used as a basis for method 1 was 70.9, also within the interval. Thus it is seen that in this case the value predicted by the use of method 2 and that used in method 1 are consistent with the interval estimate of method 3.

If a 0.95 confidence interval is desired for V_1, it is given by observed average log $V_1 \pm t_{.05}.s$, where $t_{.05}$ and s are the same values as in the preceding. Thus the 0.95 confidence interval for log V_1 calculated from the above data is 1.835±0.026; i. e. from 1.809 to 1.861, so that the 0.95 confidence interval for V_1 is 64.4—72.6.

STATISTICAL ANALYSIS OF COMPARISON EQUATION

The three methods described above for combining the results of a series of trials are based on the original comparison equation V_1: $(ADH_1)(AT_1)=V_2:(ADH_2)(AT_2)$. Since this equation occupies so important a place in analyzing the experimental data, it was decided to test its validity through statistical analysis of results obtained by its use. In order for this equation to be valid it is necessary that $\frac{V}{(ADH)\ (AT)}$ be equal to some constant which will depend on the variety concerned, over the range of time-temperature conditions likely to be met in practice.

[7] It should be noted that the probability refers to the interval including the true value of V_2. It is *wrong* to say "the probability is 0.95 that the true V_2 will fall in the interval." The true V_2 is fixed (although unknown) when the variety and the time-temperature conditions are specified. It is the interval which varies as a result of sampling fluctuations, not V_2.

If $(AHD)(AT)/(V)=k$, a constant for a given variety, then taking logarithms, this implies that $\log (ADH)+\log(AT)-\log (V)=\log k=K$, a constant independent of temperature conditions, also. Since this latter form is more convenient for statistical analysis, addition and subtraction, instead of multiplication and division, being easier to handle statistically, the analysis has been performed in terms of logarithms. From the data of preliminary trials 1 and 3 and variety trial 1, the values of $\log k$ for the Perfection variety, shown in table 8, were obtained.

TABLE 8.—*Values of log k for Perfection values from preliminary trials 1 and 3 and varietal trial 1*

Preliminary trial No. 1	Preliminary trial No. 3	Varietal trial No. 1
3.60	3.73	3.57
3.65	3.53	3.74
3.67	3.64	3.61
3.69	3.71	3.75
3.67	3.60	3.71
3.70	3.73	3.70
3.59	3.67	3.79
3.65	3.62	3.60
3.60	3.71	3.66
3.65	3.73	3.68
[1] 3.647	[1] 3.667	[1] 3.681

[1] Mean.

It can be seen that the three means differ in the second decimal place only. Using Student's t test, the comparisons P. T. 1 vs. P. T. 3, P. T. 1 vs. V. T. 1, and P. T. 3 vs. V. T. 1 yielded t values of 0.808, 1.327, and 0.449, respectively, all of which are seen to be less than the 5-percent significance level for 18 degrees of freedom (i. e., 2.101), indicating that there is no reason to suppose that $\log k$, and therefore k, is different in the three tests. In like manner, the mean values of $\log k$ for preliminary trails 1 and 3 were compared for Gradawax and Alaska—these did not occur in variety trail 1—and the resulting values of t were 1.625 and 0.108, respectively, also nonsignificant. Therefore, it appears that, so far as a given variety is concerned, the ratio is sensibly constant; at least from the data at hand, no significant departure from constancy has been detected when the temperature conditions are varied within the range of temperature conditions of the data examined.

As regards precision, method 1 is definitely less precise than methods 2 and 3 since the error of estimation of MV_2, say, by method 1 involves the sampling errors of PV_1, PV_2, and MV_1, whereas the error of estimation of MV_2 by either method 2 or method 3 involves the sampling error of MV_1 only. Estimates obtained by method 1 are, however, sufficiently accurate for general purposes. Whereas method 1 cannot be employed unless data for a check variety, here Perfection, are at hand for both trial conditions, nevertheless, when it is applicable the simplicity of the calculation is a point in its favor.

Method 3 is the only one of the three which gives a clear indication—provided in this instance by the width of the confidence interval—of the accuracy of the estimate. When method 2 has been employed, a

crude index of the accuracy of the estimate can be obtained without carrying out method 3 to completion by evaluating

Estimated geometric mean V_2=antilog (estimated average log V_2) and comparing this value with estimated average V_2 found by method 2. The estimated geometric mean V_2 will be less than the estimated average V_2 by an amount which depends on the variation among the observed V_2 values, equality being attained only when all of the observed V_2 values are equal. Thus, in table 9, it may be noted that method 2 gives 64.4 for Wisconsin Merit and for Cade and the corresponding geometric means are 54.6 and 64.1 respectively, indicating that the value for Cade is estimated more accurately than that for Wisconsin Merit. A glance at the relative widths of the corresponding confidence intervals obtained by method 3 bears out this inference.

TABLE 9.—*Comparative aphid resistance of pea varieties listed in tables 1 and 5 as calculated for standard trial conditions by the 3 prediction methods*

Variety	Method 1, average value	Method 2, average value	Geometric mean [1]	Method 3, interval
Onward	25.0	26.0	20.4	11.1 – 37.4
Pride	28.5	29.6	24.3	14.2 – 41.4
Gradawax	30.4	31.6	29.0	20.4 – 41.1
Wisconsin Penin	34.2	35.6	33.4	25.0 – 44.8
Stratagem	43.3	41.7	37.2	23.8 – 57.9
Potlatch	53.2	51.3	48.6	36.3 – 65.2
Yellow Admiral	52.7	54.8	51.2	38.6 – 68.1
Confidence (light)	56.2	55.2	53.2	43.2 – 65.6
Alaska	52.4	54.5	53.5	46.2 – 61.8
Wisconsin Merit	62.0	64.4	54.6	33.3 – 89.3
Glacier	57.8	56.8	55.3	46.6 – 65.8
Sherwood	59.2	57.1	56.2	49.7 – 63.7
Confidence (dark)	58.8	57.8	57.3	52.0 – 63.1
Horsford	60.5	59.7	59.7	54.6 – 64.4
Emerald	61.3	60.6	59.4	52.6 – 67.1
Surprise	59.2	61.4	59.4	49.0 – 72.1
Dwarf Defiance	62.8	60.6	60.0	54.6 – 65.9
Asgrow No. 83	64.0	66.5	60.5	42.5 – 86.3
English broadbean [2]	61.9	61.9	61.7	56.6 – 67.1
Canner King	63.2	62.4	62.2	58.9 – 65.8
Cade	64.3	64.4	64.1	59.4 – 69.2
Creole	66.3	66.3	66.2	64.0 – 69.2
Danby Stratagem	69.9	67.4	66.5	53.7 – 82.4
Perfection (variety trial 2)	[3] (70.9)	68.3	68.1	66.2 – 70.0
Perfection (variety trial 4)	(70.9)	69.7	69.5	66.1 – 73.1
Daniel	69.8	69.1	69.8	66.5 – 73.3
Perfection (variety trial 3)	(70.9)	70.0	69.8	66.2 – 73.6
Profusion	72.2	75.0	70.2	60.6 – 97.3
Perfection (variety trial 5)	(70.9)	71.0	70.8	66.4 – 75.5
Perfection (variety trial 1)	(70.9)	73.6	73.3	68.2 – 78.7
Perfection (preliminary trial 1)	(70.9)	73.9	73.5	69.2 – 78.0
Asgrow No. 40	71.7	74.4	73.6	65.8 – 82.4
Fasciated Sweet	75.7	74.9	74.0	69.8 – 78.3
Prince of Wales	73.6	76.5	75.9	68.6 – 84.0
Early Wales	74.7	77.6	77.3	69.7 – 85.7
Triumph	83.4	86.6	86.5	81.8 – 91.4

[1] Geometric mean=antilogarithm of average log V.
[2] *Vicia faba.*
[3] Estimated value.

It is recommended, therefore, that the method to be used be determined by the degree of accuracy desired. In a general testing program where a quick and easy method will suffice to show the relative resistance of varieties tested, methods 1 and 2 are sufficiently accurate. In technical studies where a certain degree of confidence is desired, method 3 should be used.

558507—43——2

EXTENT OF APHID RESISTANCE IN PEAS

The known range of aphid resistance in peas is small. The most resistant pea varieties found to date are but midly resistant when compared, for example, with certain alfalfa lines (*2*), (*6*) which are almost immune to attack by the pea aphid. When compared with susceptible pea varieties, nevertheless, the difference is large enough to be of significance in the field.

Some 50 varieties of peas have been tested with the greenhouse technique. Three trials, comprising 18 varieties were discarded because of high temperature and consequent mortality of parent aphids. Varieties tested in preliminary trials 1 and 2 and in the 5 varietal trials were placed in their approximate positions in the resistance-susceptibility scale by means of the 3 methods described above. Values given the varieties in each of the 3 resultant scales are based on the probable response of these varieties under a standard set of experimental conditions, namely, 4,750 accumulated degree-hours at an average temperature of 68.0° F. No trials were run at exactly these standard conditions, so that no value was available for the Perfection check variety for these conditions. The value was determined, therefore, by averaging the hypothetical values predicted for the standard conditions by means of method 2 using the data from preliminary trial 1 and the 5 varietal trials. The value, 70.9, was obtained by this procedure. The results are presented in table 9.

Values predicted for the 31 varieties in table 9 by each of the three prediction methods are found to fall in approximately the same relative position, with respect to each other, in the resistance-susceptibility scale. All values obtained by the use of methods 1 and 2 fall within the interval necessary for 95 percent confidence as determined by the use of method 3. In fact, these values fall in the approximate center of the interval in the majority of instances. As noted above, the geometric mean predicted by method 3 will never exceed the corresponding arithmetic mean obtained by the use of methods 1 and 2. The size of the interval necessary for 95 percent confidence varies considerably from variety to variety depending upon the uniformity of progeny counts. In the case of Wisconsin Merit, Asgrow 83, and Profusion, the presence of some extremely low counts resulted in intervals much too large for practical application.

SUMMARY AND CONCLUSIONS

A new technique for detecting the presence and measuring the magnitude of aphid resistance in peas has been described. The principal advantages of the procedure are (1) accuracy under variable environal conditions, (2) rapidity, and (3) adaptability to requirements of the pea breeder. While the procedure is of value in supplying an efficient and rapid means of evaluating the comparative aphid resistance of established pea varieties and strains, its greatest promise lies in its ability to detect resistance in individual plants belonging to segregating families. The inability of pea breeders in the past to determine the comparative aphid resistance of single plant selections has been one of the principal factors in preventing the development of new pea varieties having increased resistance to the aphid. No variety of pea has yet been found that exhibits a high degree of resist-

ance to the aphid, and for this reason it seems unlikely that new varieties of greatly superior resistance will be developed in the near future. Nevertheless, the incorporation of partial resistance, such as is found in the Onward variety, into other peas having more desirable canning qualities doubtless would be of real value to growers and packers of this staple crop.

LITERATURE CITED

(1) BARTLETT, M. S.
1937. SOME EXAMPLES OF STATISTICAL METHODS OF RESEARCH IN AGRICULTURE AND APPLIED BIOLOGY. Roy. Statis. Soc. Jour. Sup. 4: 137–170, illus.

(2) BLANCHARD, R. A., and DUDLEY, J. E., JR.
1934. ALFALFA PLANTS RESISTANT TO THE PEA APHID. Jour. Econ. Ent. 27: 262–264.

(3) FISHER, R. A.
1938. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 7, 356 pp., illus. Edinburgh and London.

(4) HARRINGTON, C. D.
1941. INFLUENCE OF APHID RESISTANCE IN PEAS UPON APHID DEVELOPMENT, REPRODUCTION, AND LONGEVITY. Jour. Agr. Res. 62: 461–466, illus.

(5) MALTAIS, J. B.
1937. RESISTANCE OF SOME VARIETIES OF PEAS TO THE PEA APHID ILLINOIA PISI KALT. Ontario Ent. Soc. Ann. Rpt. (1936) 67: 40–45, illus.

(6) PAINTER, R. H., and GRANDFIELD, C. O.
1935. PRELIMINARY REPORT OF RESISTANCE OF ALFALFA VARIETIES TO PEA APHIDS ILLINOIA PISI KALT. Amer. Soc. Agron. Jour. 27: 671–674, illus.

(7) SEARLS, E. M.
1932. A PRELIMINARY REPORT ON THE RESISTANCE OF CERTAIN LEGUMES TO CERTAIN HOMOPTEROUS INSECTS. Jour. Econ. Ent. 25: 46–49.

(8) ———
1935. THE RELATION OF FOLIAGE COLOR TO APHID RESISTANCE IN SOME VARIETIES OF CANNING PEAS. Jour. Agr. Res. 51: 613–619, illus.

COMPARATIVE ABILITY OF SEVERAL SPECIES OF LYGUS AND THE SAY STINKBUG TO DAMAGE SUGAR BEETS GROWN FOR SEED [1]

By ORIN A. HILLS

Associate entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

In earlier work [2] the writer has shown that *Lygus hesperus* Knight and *L. oblineatus* (Say) and two of the stinkbugs—*Chlorochroa sayi* Stål and *Thyanta custator* (F.)—may render nonviable the seeds of sugar beets (*Beta vulgaris* L.) grown for seed. These two species of *Lygus* and also *L. elisus* Van Duzee occur in the seed-beet fields of the Southwestern States. The same species do not predominate in all districts, however, and field observations have indicated that all species may not cause equal damage. An investigation was therefore undertaken at Phoenix, Ariz., in 1940 to determine the potential as well as the comparative ability of the various forms of *L. hesperus*, *L. oblineatus*, and *L. elisus* to reduce the viability of seed balls of sugarbeets grown for seed, and to compare this damage with that caused by adults of *Chlorochroa sayi*.

MATERIALS AND METHODS

In December 1939 three 16-mesh screen cages measuring 10 by 15 by 7 feet were placed in a sugar-beet field over groups of plants to protect them from infestation by *Lygus* and other seed-feeding insects (fig. 1). At this time very few insects were present, but to insure insect-free conditions the plants within the cages were sprayed with a strong mixture of pyrethrum-in-oil.

The following April, after the plants had developed seedstalks, sleeve cages approximately 7 inches in diameter and 14 inches long were tied on individual spikelets of the plants within the large cages. These sleeve cages were made of curtain scrim having approximately 32 meshes to the inch, which is sufficiently fine to retain even small *Lygus* nymphs. The cages were set up in randomized blocks, 11 cages constituting a block, and each block was replicated 10 times. A sufficient number of flowers were included in each cage to develop from 300 to 400 seed balls, which was considered an excess of food for the insects. A single insect was then introduced into each cage so that each block would contain 1 female, 1 male, and 1 nymph of *L. hesperus*, *L. oblineatus*, *L. elisus*, and a *Chlorochroa sayi* adult without respect to sex; and 1 cage was maintained insect-free as a check. All the cages of 1 block were placed on the same beet plant to avoid any possible differences between plants. Figure 2 shows a close-up of 1 of the blocks within the screen cage.

[1] Received for publication November 25, 1942.

[2] HILLS, O. A. ISOLATION-CAGE STUDIES OF CERTAIN HEMIPTEROUS AND HOMOPTEROUS INSECTS ON SUGAR BEETS GROWN FOR SEED. Jour. Econ. Ent. 34: 756-760, illus. 1941.

Insects for this work were reared as pure-species colonies in cloth-covered cages measuring 4 by 5 by 7 feet, shown at the extreme left in figure 1. For the tests of nymphal damage nymphs were taken directly from these colony cages and placed in the sleeve cages. Nymphs as nearly as possible in the second instar were used and allowed to mature within the cage. The newly emerged adults were removed immediately and replaced with other second instars of the same species. For tests of adult damage individually reared specimens were used in order to prevent reproduction within the cages containing females. Adults were obtained by placing large nymphs

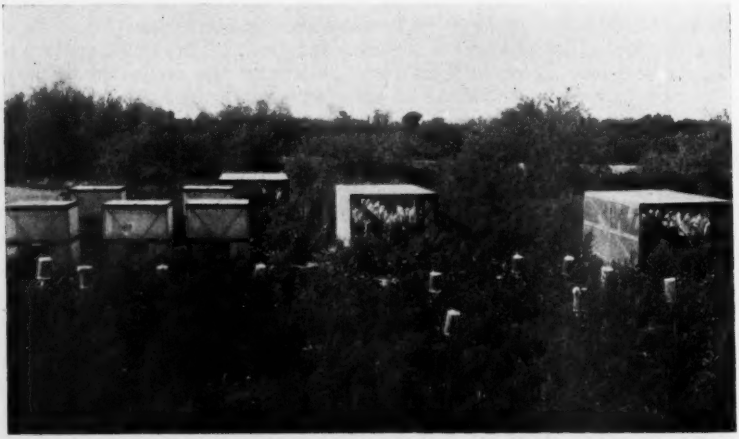

FIGURE 1.—Cages used in insect-isolation studies. The large cloth-covered cages at the left contained pure-species colonies. The three large screen cages were for protection of the plants from outside infestation; the small sleeve cages within contained the insects being tested. The cylindrical screen cages in the foreground were not used in this experiment.

from the colony cages in individual rearing cages on beet spikelets (fig. 3). As adults emerged, males and females were placed in separate sleeve cages. The insects were observed every 2 or 3 days, and dead individuals were replaced with live specimens.

The insects were maintained on the plants from the beginning of the blooming period until the seed matured, which in most cases was from May 10 to June 24. The average number of individuals used in maintaining one insect in each cage during this period was: For *Lygus hesperus*, females 1.4, males 2.1, and nymphs 3.9; for *L. oblineatus*, females 1.3, males 2.1, and nymphs 3.9; for *L. elisus*, females 1.4, males 1.9, and nymphs 4.9; for adults of *Chlorochroa sayi*, 1.4.

When the seed was mature, the sleeve cages were brought into the laboratory, where the seed balls [3] were stripped from the spikelets by hand and screened over a $\frac{7}{64}$-inch mesh hand screen to remove trash and small seed balls. The seed balls from each cage were then counted and germination analyses made to determine their viability.

[3] In this paper the term "seed ball" is used to denote the dried fruit (diclesium) containing one or more true seeds, and is synonymous with the term "beet seed" in commercial usage.

Since it was desirable to know whether the insects were capable of feeding on one seed without damaging other seeds in the same ball, or whether they rendered the entire ball nonviable, counts were made not only of viable balls but also of the sprouts developing from each ball.

FIGURE 2.—Close-up of one of the blocks of sleeve cages, within one of the large screen cages.

RESULTS

The results of the tests are summarized in table 1. There was a significant increase in the number of nonviable seed balls in all cages containing insects over that in the check cages except in the cages containing *Lygus elisus* males. More nonviable seed balls were found in the *Chlorochroa sayi* cages than in any of the *Lygus* cages. There was a definite reduction in the number of sprouts per viable ball only in the case of damage by *C. sayi*. A slight reduction is indicated for *L. hesperus* nymphs, but in general feeding by *Lygus* spp. rendered all the seeds within the ball nonviable, whereas in cages containing *C. sayi* one or more of the seeds in certain balls were damaged but the other seeds within the ball germinated.

FIGURE 3.—Close-up of individual rearing cages, tied on tips of sugar-beet spikelets.

TABLE 1.—*Viability of seed balls from sugar-beet spikelets confined in cages with individuals of Lygus spp. and Chlorochroa* sayi; *means of 10 replicate cages*

Insect	Seed balls per cage			Sprouts per viable ball per cage
	Nonviable	Viable	Total	
Lygus hesperus:	*Number*	*Number*	*Number*	*Number*
Female	96.8	344.4	441.2	1.48
Male	62.8	370.3	433.1	1.53
Nymph	114.4	325.1	439.5	1.46
Lygus oblineatus:				
Female	96.7	325.5	422.2	1.56
Male	65.1	383.8	448.9	1.58
Nymph	79.0	324.5	403.5	1.61
Lygus elisus:				
Female	65.1	360.7	425.8	1.58
Male	54.0	355.2	409.2	1.56
Nymph	65.5	365.8	431.3	1.69
Chlorochroa sayi	206.1	126.7	332.8	1.27
None (check)	23.4	425.1	448.5	1.62
Difference required for significance	36.0	----------	[1]	.15

[1] Not significant by the *F* test.

Since the cages were placed on the plants when they were in the very early blossom stage, it was impossible to encage a definite number of seed balls, and the only way to approach uniformity in this regard was to encage approximately the same length of each spikelet. However, the fact that there were no significant differences between treatments in total number of seed balls produced per cage indicates that none of these insects cause a reduction in the number of seed balls produced, which is in accordance with previous experiments.

Since the number of viable seed balls remaining in the *Lygus* cages was far in excess of the number of nonviable seed balls, it is probable that the damage caused by these insects had reached its maximum, and that no more nonviable seed balls would have been produced if larger numbers had been present. The number of viable seed balls remaining in the *Chlorochroa sayi* cages was considerably smaller than in the *Lygus* cages, and if these cages had contained more seed balls the number of nonviable balls might have been greater.

The mean differences between the number of nonviable seed balls occurring in cages containing the various forms and species of *Lygus* become more evident when the data pertaining to *Lygus* are analyzed without the data from the *C. sayi* or check cages. Table 2, in which the different forms of *Lygus* are grouped without respect to species, shows that the females and nymphs cause an equal amount of damage, but somewhat more than that caused by the males. Table 3, showing the same data for the three species of *Lygus* without respect to form, indicates that *Lygus elisus* damages a significantly smaller number of seed balls than does either *L. hesperus* or *L. oblineatus*.

TABLE 2.—*Viability of sugar-beet seed balls produced in cages containing males, females, and nymphs of Lygus without respect to species*

Form	Seed balls per cage	
	Nonviable	Viable
	Number	*Number*
Adult female	86.2	343.5
Adult male	60.6	369.8
Nymph	86.3	338.5
Difference required for significance	15.2	

TABLE 3.—*Viability of sugar-beet seed balls produced in cages containing various species of Lygus without respect to form*

Species	Seed balls per cage	
	Nonviable	Viable
	Number	*Number*
Lygus hesperus	91.3	346.6
Lygus oblineatus	80.3	344.6
Lygus elisus	61.5	360.6
Difference required for significance	15.2	

The results of these experiments not only furnish a basis for comparing the amount of damage to sugar-beet seed attributable to the various species and forms of *Lygus* as well as to adults of *Chlorochroa*

sayi, but also, since they are based on the actual number of seed balls damaged, they give some idea of the damage to be expected from each individual occurring in the field. The fact that *Lygus* nymphs cause as much damage as the females is of prime importance, since at certain seasons the nymphs are far more numerous in the fields than the adults. The fact that *L. elisus* causes less damage than the other two species is also important in evaluating field populations of *Lygus*.

Less emphasis has been placed on *Chlorochroa sayi*, since this insect is usually present in beet fields in much smaller numbers than *Lygus*. In certain years, however, *C. sayi* has been known to occur in some areas in large numbers with disastrous results. This can easily be understood by the results of this experiment, which show that a single specimen maintained on the plant during the fruiting period is capable of destroying many more seed balls than any of the species of *Lygus*.

SUMMARY

This paper reports the results of a study to determine the extent of damage that may be caused to sugar beets grown for seed by several forms of three species of *Lygus* that occur in the seed-beet fields of the Southwestern States and by adults of *Chlorochroa sayi* Stål. The experiments were conducted with insects caged individually on seed-beet plants grown within large screen cages. *L. hesperus* and *L. oblineatus* caused more damage than *L. elisus*. Nymphs caused as much damage as females, and both nymphs and females caused more damage than males. Adults of *C. sayi* caused more damage than any of the species or forms of *Lygus*.

INFLUENCE OF VARIOUS FACTORS ON THE STARCH CONTENT OF KANSAS-GROWN POTATOES AND SWEETPOTATOES [1]

By H. N. BARHAM, *industrial chemist, Kansas Agricultural Experiment Station;* GEORGE KRAMER and G. NATHAN REED, *Department of Chemistry, Kansas State College*

INTRODUCTION

In addition to its value as a food material, the potato (*Solanum tuberosum* L.) has achieved considerable commercial importance because of the desirability of its starch for use in the textile industry. Recent studies (*8*)[2] have indicated that starch from sweetpotato (*Ipomoea batatas* (L.) Lam.) may also be of industrial value and an experimental mill has been in operation at Laurel, Miss., since 1934.

Systematic studies (*4, 6, 9, 10, 11*) of the starch content of both the potato and the sweetpotato have been made for a number of localities. These studies have revealed a rather wide variation in starch content. The results of a similar study of Kansas-grown potatoes and sweetpotatoes are reported in the present paper. For this work a series of carefully selected, representative samples was obtained. Since it was known that starch content may be influenced by a number of factors, the samples were selected in such a manner that as many of these factors as possible might be studied. However, the choice of samples was limited by the fact that they were collected by one man in the course of his regular duties.

The following factors were believed to affect the starch content of potatoes: Variety, stage of maturity, soil, previous crop grown on the land, and storage. Subsequent paragraphs will indicate the way in which these factors were taken into consideration during the course of the work.

The Irish Cobbler, Warba, and Bliss Triumph potatoes were selected because they are the varieties most commonly grown in Kansas.

The regular harvest season for Kansas in 1939 was July 11–14. Samples taken from the ground prior to July 11 were immature and were labeled "early harvest." Samples taken 5 to 10 days after the regular harvest had been completed were designated "late harvest." No record of the previous crop was obtained except in those instances specifically mentioned.

Two identical samples of Irish Cobbler were used to study the effect of storage. One sample was placed in cold storage (4.4° C.) and the other was stored in a shed. Weight changes as well as starch content were determined for each sample at monthly intervals for a 6-month period.

For sweetpotatoes the following factors were studied: Variety, locality, stage of maturity, and curing. The Little Stem Jersey, Big Stem Jersey, Improved Big Stem Jersey, Red Bermuda, and Nancy

[1] Received for publication November 5, 1942. Contribution No. 262 of the Department of Chemistry, Kansas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 406.

Hall were selected as varieties common to Kansas. Samples taken before October 1 were regarded as "early harvest"; those taken after that date were considered as "regular harvest." Five samples of regular harvest potatoes were put through the curing process which consisted of heating in a kiln at 26.7° to 32.2° C. for 10 to 14 days.

EXPERIMENTAL MATERIALS AND METHODS

COLLECTION OF SAMPLES

Thirty-six samples—18 of potatoes and 18 of sweetpotatoes—were collected during the period July to November 1939.[3] They were shipped to Manhattan, Kans., where they were placed in storage, the potatoes at 4.4° C. and the sweetpotatoes at room temperature, until used for the preparation of the analytical samples or the extraction of starch. Not more than 60 hours (average about 36) elapsed between the digging of a sample and the time it reached storage. The analytical samples were prepared in less than 24 hours after the samples were received; starch was removed from corresponding samples soon thereafter. A detailed description of the samples is given in tables 1 and 2.

TABLE 1.—*Data on field samples of Kansas-grown potatoes collected for analysis as to starch content*

Sample No.	Variety	Source	Type of soil	Stage of harvest	Starch (fresh weight basis) obtained when analyzed by—	
					Acid hydrolysis	Diastase hydrolysis
					Percent	*Percent*
1	Irish Cobbler	Near Edwardsville	Sandy loam	Early	[1] 13.26	11.77
4	do [2]	Newman Experimental Fields, Newman.	Fine sandy loam	do	14.67	13.40
5	do [3 4]	do	do	Regular	14.31	12.55
8	do [2]	do	do	do	15.41	13.84
9	do [5]	do	do	do	14.64	13.22
10	do [2]	Near Loring	Loamy sand	do	13.28	11.86
11	do [6 7]	Near Manhattan	Sandy loam	do	14.67	13.28
12	do [7 8]	do	do	do	14.67	13.28
13	do	Near Edwardsville	do	Late	12.18	10.99
16	do	Newman Experimental Fields, Newman.	Fine sandy loam	do	13.30	11.98
17	do	Near Newton	Loamy sand	do	12.46	11.15
18	do [3]	Near Atchison	Upland	do	15.19	13.25
2	Bliss Triumph	Near Linwood	Sandy loam	Early	10.99	9.90
6	do [3 4]	Newman Experimental Fields, Newman.	Fine sandy loam	Regular	11.45	9.90
15	do	Near Linwood	Sandy loam	Late	9.81	8.80
3	Warba	Near Edwardsville	do	Early	12.59	11.17
7	do [3 4]	Newman Experimental Fields, Newman.	Fine sandy loam	Regular	13.56	11.90
14	do	Near Edwardsville	Sandy loam	Late	11.82	10.51

[1] Because of unsatisfactory handling, this sample will be disregarded in the discussion.
[2] From land on which potatoes were grown the previous year.
[3] Used in variety comparison.
[4] From land on which oats (followed by sweetclover, which was a failure) was grown the previous year.
[5] From land on which alfalfa was grown the previous year.
[6] Used in cold-storage (4.4° C.) experiment.
[7] Graded: Grade 2.
[8] Used in shed-storage experiment.

[3] The samples were collected by Dr. O. H. Elmer of the Department of Botany, Kansas State College, in connection with his field work among the potatoe growers of the State.

TABLE 2.—*Data on field samples of Kansas-grown sweetpotatoes collected for analysis as to starch content*

Sample No.	Variety	Source	Stage of harvest	Starch (fresh-weight basis) obtained when analyzed by—	
				Acid hydrolysis	Diastase hydrolysis
				Percent	*Percent*
1	Little Stem Jersey	Kansas River Valley	Early	15.21	13.44
4	do	do	Regular	16.88	15.13
5	do	do	do	16.57	14.78
16	do	do	do.[1]	15.19	13.17
17	do	do	do.[1]	15.33	13.13
8	do	Arkansas River Valley	do	17.39	15.45
11	do	do	do	18.84	16.74
2	Nancy Hall	Kansas River Valley	Early	21.06	19.45
13	do	do	Regular	21.50	19.42
18	do	do	do.[1]	18.96	16.59
7	do	Arkansas River Valley	do	21.49	19.73
9	do	do	do	19.69	17.93
3	Red Bermuda	Kansas River Valley	Early	13.84	12.85
12	do	do	Regular	14.50	13.57
14	do	do	do.[1]	14.87	13.42
6	Improved Big Stem Jersey	do	do	18.69	16.79
15	do	do	do[1]	17.83	15.60
10	Big Stem Jersey	Arkansas River Valley	do	18.94	17.14

[1] Cured sample.

SHED STORAGE VERSUS COLD STORAGE

Potato samples Nos. 11 and 12 were used in the storage experiments. The samples were identical in nature—Irish Cobbler, regular harvest, grade 2—and three 100-pound sacks were used for each experiment. Sample No. 11 was stored in a commercial cold storage plant in Manhattan, while sample No. 12 was placed in a shed of light frame construction, elevated on pilings to a height of about 5 feet above the ground. Of the three sacks of potatoes used in each experiment, one, designated "weight sample," was used only for determining the changes in weight (table 3) that occurred during the experiment;

TABLE 3.—*Weight record on storage samples*

Month	Cold storage			Shed storage		
	Weight	Total loss		Weight	Total loss	
	Pounds	*Pounds*	*Percent*	*Pounds*	*Pounds*	*Percent*
July	90.0			89.5		
August	86.0	4.0	4.4	77.5	12.0	13.4
September	83.5	6.5	7.2	69.0	20.5	22.9
October	83.0	7.0	7.8	62.5	27.0	30.2
November	82.5	7.5	8.3	60.5	29.0	32.4
December	81.5	8.5	9.4	59.0	30.5	34.1
January	80.0	10.0	11.1	58.5	31.0	34.6

the second was used as the source of the analytical samples, and the third was held in reserve. On July 14, the weight of each weight sample was recorded and 10 pounds of potatoes were removed from the second sack of Nos. 11 and 12 for the preparation of the analytical samples. This procedure was repeated on the fourteenth of each

succeeding month to and including January 14, 1940. The analytical samples were handled in a manner subsequently described and analyzed with the other potato samples.

During the 6-month period the potatoes in shed storage were exposed to a wide range of conditions. While no attempt was made to keep a daily record of the temperature and humidity fluctuations, the monthly average for each of these factors as recorded by the Kansas State College weather observers is shown in table 4. The conditions

TABLE 4.—*Average temperature and humidity during the period of shed storage*

Month	Average mean temperature	Average relative humidity
1939	°F.	*Percent*
July	83.75	33.5
August	75.87	64.9
September	76.31	45.7
October	61.77	51.4
November	44.83	64.8
December	39.01	61.1
1940		
January	13.76	66.2

to which the sample in cold storage was exposed were fairly constant. The company operating the storage plant stated that the temperature was maintained at 4.4° C. (±1°) and that previous determinations of relative humidity gave an average of about 85 percent for that part of the plant in which the samples were kept.

PREPARATION OF ANALYTICAL SAMPLES

Ten pounds of potatoes were taken from each of the incoming samples, scrubbed with a vegetable brush to remove all foreign matter, and dried by spreading on a wire screen before an electric fan. They were then cut into small pieces and passed four times through a large, dry meat grinder. No water was added at any stage of the preparation. The thick watery suspension was thoroughly mixed, 100 gm. weighed out on an analytical balance, and added to sufficient hot, acid-free, 95 percent ethyl alcohol to make the final alcoholic concentration—allowing for the water content of the potato—about 80 percent. Approximately 15 minutes was required for grinding and weighing the samples. The alcoholic suspension, contained in a fruit jar, was heated on a water bath for 30 minutes at a temperature slightly below the boiling point. The contents of the jar were thoroughly stirred at frequent intervals during the heating. The jars were then sealed, labeled, and stored until the next step in the preparation of the samples could be carried out.

Each sample was carefully filtered from the alcohol with suction on a Hirsch funnel. The residue was washed with about 150 cc. of 80-percent acid-free alcohol and dried as thoroughly as suction would permit. The funnel, containing filter paper and sample, was placed in an electric oven at 80° C. and kept there for 14 hours. The sample was carefully transferred to a tared weighing bottle, dried for an additional 3 hours at 80°, cooled in a desiccator, and weighed. This

was taken as the weight of dry material that was obtained from 100 gm. of potatoes and that was insoluble in 80-percent alcohol. The dried material was ground to a fine powder and stored until needed for analysis. For the actual analysis, a small sample of each of these dry powders was placed in a tared weighing bottle, dried again at 80° for 2 to 3 hours, cooled in a desiccator, and reweighed.

METHOD OF ANALYSIS

A number of methods have been described as suitable for the determination of starch in vegetables. In most cases, satisfactory checks can be obtained as long as a given procedure is followed but rarely do the results obtained by any two methods check satisfactorily. The Association of Official Agricultural Chemists recommends two methods (*1, p. 342*). Both of these were used and the starch content is reported as determined by Direct Acid Hydrolysis, A. O. A. C. XXVII, 31, and by the Diastase Method with Subsequent Acid Hydrolysis, A. O. A. C. XXVII, 32.

The diastase used in the second method was Diastatic Spray Dried Malt No. 25, supplied by Eimer and Amend. This preparation contained 2 percent moisture. An infusion of the malt was prepared as needed. For each 80 cc. of malt extract required, 5 gm. of the malt powder was stirred for 20 minutes with water at room temperature. The extract was filtered and the clear brown infusion was used according to the directions given. Blank determinations on the enzyme preparations were run at frequent intervals and the experimental data corrected accordingly.

CALCULATION OF RESULTS

Table 5 shows a typical treatment of data obtained from the analysis of a sample of potato by the use of the direct acid hydrolysis method. In this case, the weight of the dry sample was 17.7798 gm.

TABLE 5.—*Sample of analytical data obtained in analysis of potato by the use of the direct acid hydrolysis method*

Weight of sample (grams)	$Na_2S_2O_3$	Cu_2O	Dextrose	Starch		
				Total	Dry weight	Fresh weight
	Cc.	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent* [1]
1.5838	13.8	149.0	65.40	58.86	74.33	13.215
	13.9	150.1	65.94	59.35	74.95	13.325
	13.8	149.0	65.40	58.86	74.33	13.215
	13.9	150.1	65.94	59.35	74.95	13.325
1.6583	14.4	155.5	68.40	61.56	74.25	13.201
	14.5	156.6	68.90	62.01	74.79	13.297
	14.6	157.6	69.34	62.41	75.27	13.383
	14.5	156.6	68.90	62.01	74.79	13.297

[1] Average, 13.283.

In all cases, an accuracy of 0.2 cc. of standard sodium thiosulfate was required for the titrations to be accepted, and when calculating the averages only those values in which the percentage of starch (calculated to the original potato) checked within 0.2 percent were considered.

PRESENTATION AND DISCUSSION OF RESULTS

COMPARISON OF THE ANALYTICAL METHODS

There are several methods which have been used to determine starch on a quantitative basis and, while these methods may agree within themselves, they do not check with each other. Hence, it may be assumed that none of the known methods is an absolute measure of the starch content of any material. The two methods used in this work are accepted as probably the most accurate for this type of analysis. Since both methods were used with the same samples and under comparable conditions, an opportunity is presented to compare them.

The results obtained by the direct acid hydrolysis method were always higher than those obtained by the diastase method. In any given series of determinations the two sets of data showed the same trend—that is, an increase (or decrease) in starch content was reflected in the results obtained by both methods—but the values obtained by the two methods for any given sample did not agree. In the case of the potatoes, the starch content as determined by acid hydrolysis averaged 1.39 percent higher than the values obtained by the diastase method. The greatest difference observed was 1.94 percent, the least was 1.01 percent. With the cold-storage samples the range was from 1.12 to 1.49 percent with an average difference of 1.33 percent. The shed-storage samples showed a range of 1.21 to 1.59 percent, or an average of 1.43 percent. Considering all samples of potatoes the average difference was about 1.4 percent. The difference obtained when the sweetpotatoes were analyzed was noticeably greater, the average being 1.81 percent with a range of 0.93 to 2.37 percent.

Differences due to the method employed were expected since it has been generally accepted that the acid hydrolysis method may bring about the hydrolysis of substances other than starch while the enzyme diastase is thought to be relatively specific for starch. In this connection, however, a recent publication by Balch and Phillips (*2*) reports that malt diastase acts on certain nonstarch constituents of plant materials which are usually reported as starch.

It was believed, therefore, that further differences between the two methods would become apparent if they were used to estimate the starch values of pure starch samples. Hence, samples of pure starch were prepared from the sweetpotato and potato samples, analyzed by both methods, and the results compared with the analysis of the corresponding potatoes (table 6).

TABLE 6.—*Data for comparison of the methods of analysis*

Sample	Starch (dry-weight basis) obtained when analyzed by—		Difference	Deviation
	Acid hydrolysis	Diastase hydrolysis		
Sweetpotato No. 13:	*Percent*	*Percent*	*Percent*	*Percent*
Pure starch	86.50	81.05	5.45	6.7
Sweetpotato sample	78.20	70.64	7.56	10.7
Potato No. 11:				
Pure starch	79.57	75.09	4.48	6.0
Potato sample	76.42	69.14	7.28	10.5

These data confirm the prevalent opinion that there is a real difference inherent in the two methods of procedure. More specifically, they indicate that acid promotes more complete hydrolysis of starch than does diastase and that acid is more active than diastase in the hydrolysis of substances other than starch present in the tissues of potatoes and sweetpotatoes.

It is commonly assumed that the hydrolysis of starch, by either method, produces dextrose which is measured by the reduction of Fehling's solution. In the case, however, of incomplete hydrolysis, it is probable that maltose or some of the simpler polysaccharides would be present. Further, it is possible that under the conditions of the analysis—particularly if the reducing value of the hydrolyzed product were not determined immediately—a resynthesis of dextrose into some other disaccharide, or even simple polysaccharide, might occur. All of these substances might be reducing substances but their reducing index would certainly be lower than that of dextrose. Since the diastase method gave the lower values when used on the pure starch samples it appears that the carbohydrates present were not all in the form of glucose when the reduction of the Fehling's solution was carried out. Subsequent to the completion of these experiments, Etheredge (*5*) reported similar results with other pure starches. Not only did he find that these two methods yield different results but also that there was a lack of consistency with respect to which would give the larger and which the smaller starch values. In his experiments, the acid hydrolysis method indicated a 2.4 percent higher starch value for potato starch than did the diastase-acid method; this corresponds to a deviation of 6.0 percent in the present experiments.

After analysis of all the samples had been completed, rechecks were run in certain instances. In all cases it was much easier to duplicate former results when the acid hydrolysis method was used. No difficulty was encountered in obtaining checks within 0.1 percent, while with the diastase method the best that could be obtained was 0.2 percent.

Furthermore, when the diastase method is used it is necessary to run a blank determination on the malt extract and correct the titration values accordingly. In each instance the diastase solutions were prepared in exactly the same manner and a blank was made for every eight analyses. However, it is possible that slight variations in preparing or using the diastase solutions might produce a considerable difference in the degree of hydrolysis and it may be that a blank should accompany every determination.

After considering the reasons set forth above, it was concluded that the results obtained by the direct acid hydrolysis were the more satisfactory for making comparisons. Therefore, only these data will be considered in the remainder of the discussion.

INFLUENCE OF STORAGE ON STARCH CONTENT OF POTATOES

The potato storage results obtained in these experiments (table 7) are in general agreement with previous findings pertaining to effects

TABLE 7.—*Changes in weight and starch content of Kansas-grown potatoes during a 6-month period in cold storage and shed storage*

Month	Weight of solids	Starch			Weight loss of potatoes	Solids (fresh-weight basis)	Starch (fresh-weight basis)
		In solids	When analyzed by acid hydrolysis	When analyzed by diastase hydrolysis			
	Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Percent*
COLD STORAGE							
July	19.20	76.41	14.67	13.28		19.20	14.67
August	17.49	74.34	13.00	11.77	4.4	16.75	12.43
September	17.87	71.48	12.86	11.53	7.2	16.82	12.06
October	17.60	72.29	12.72	11.25	7.8	16.33	11.73
November	16.59	71.61	11.93	10.67	8.3	15.32	10.93
December	15.86	69.39	11.06	9.59	9.4	14.49	10.04
January	15.72	67.56	10.68	9.56	11.1	14.15	9.57
SHED STORAGE							
July	19.20	76.41	14.67	13.28		19.20	14.67
August	19.36	75.16	14.55	13.34	13.4	17.07	12.60
September	20.89	74.75	15.63	14.17	22.9	17.00	12.05
October	20.24	74.69	15.11	13.56	30.2	15.53	10.54
November	19.54	74.83	14.66	13.24	32.4	14.62	9.73
December	20.44	74.93	15.35	13.86	34.1	15.25	10.12
January	21.20	73.60	15.79	14.20	34.6	15.74	10.32

of temperature and humidity changes on weight losses and on sugar-starch interconversion. The potatoes kept in cold storage over the 6-month period showed continuous and relatively gradual weight losses; the total loss was approximately 11 percent. The potatoes held in shed storage, which were subjected to radical changes in temperature and relative humidity, suffered continuous losses in weight but the rate of loss fell off markedly toward the end of the storage; the total loss was approximately 35 percent.

It may be shown graphically that changes in starch, alcohol-insoluble solids, and starch in solids from cold-storage potatoes follow approximately the same trend although there is some reason to believe that these values have responded to the variable humidity of storage. A similar treatment of shed-storage data shows a definite correlation between the changes in starch and solids. This relationship is not due, as might be expected, to a similar variation in the starch content of the solids, since there is little change in the latter between the second and fifth months. When starch values are corrected to the original weights of the potatoes, it becomes evident that there is little to choose between cold storage and shed storage, judging from the over-all losses of starch as determined by analysis; in either case, there has been a drop of from 4 to 5 percent.

One interesting relationship, more or less incidental to the practical aspects of the problem, is that observed between the starch or solids content and the water content of shed-storage potatoes as reflected by the temperature, or even better by the temperature-relative humidity ratio, at the time of sampling. Figure 1 shows graphically how changes in starch correspond to sharp changes in this ratio.

It is believed that this relationship may be explained on the basis of a dependence of the tissue-water concentration on environmental factors that affect transpiration. Other principal factors which may produce an increase in tissue-water concentration are the sugar-

starch conversion and respiration. However, the latter changes are opposing reactions in the sense that each is dependent upon the sugar concentration. It is conceivable that there may be conditions under which the relative rates of change in the sugar-starch conversion and respiration may be such that there is relatively little sugar available for respiration. In such a case, an appreciable part of the water replacing that lost through transpiration would come through the conversion of sugar to starch. With subsequent lowering of the temperature-relative humidity ratio, the tissue-water concentration

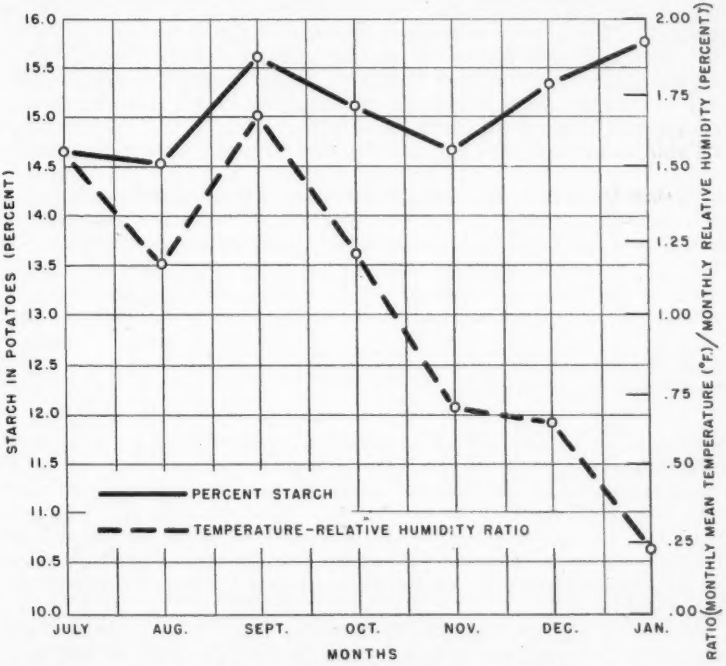

FIGURE 1.—Changes in the starch content of potatoes and in the temperature-relative humidity ratio during shed storage.

could increase through respiration and, as it does, permit a proportionate shift from starch to sugar. In such a manner, starch could increase or decrease with change in the temperature-relative humidity ratio.

The above explanation is supported in some measure by evidence recently presented by Barker (*3*) that, in potatoes, sucrose either is or determines the respirable substrate. Barker found that, within the temperature limits of 1° to 10° C. and excepting high sucrose concentrations, curves relating the rate of respiration with the concentration of extractable sucrose closely resemble those which characterize the rate of reaction-substrate concentration of enzymic reactions in vitro. Although it must be recognized that higher temperatures might alter the form of respiration-substrate curves, presumably be-

cause of lower sugar concentrations, it should be manifest that the water concentration plays an important part in the balance mechanism existing in plant tissue whatever the prevailing conditions may be. Accordingly, those environmental factors which may influence the tissue-water concentration may indirectly affect the sugar-starch balance in the tissue.

INFLUENCE OF VARIETY, HARVEST PERIOD, AND SOIL TYPE ON STARCH CONTENT OF POTATOES AND SWEETPOTATOES

The starch content of the various varieties is well illustrated by the data obtained on samples 6, 7, and 8 (shown in the middle column of table 8). These were samples grown under the same conditions, on the same soil, and harvested at the regular period. Inspection of table 8 shows that the Irish Cobbler had the greatest starch content, with Warba next and Bliss Triumph the least. This same trend may be observed throughout the other samples used.

Table 8 also shows the effect of the harvest period on starch content.

TABLE 8.—*Effect of variety and harvest period on starch content of potatoes*

Variety	Starch (fresh-weight basis) when harvest was—		
	Early	Regular	Late
	Percent	*Percent*	*Percent*
Irish Cobbler	14.67	15.41	13.30
Warba	12.59	13.56	11.82
Bliss Triumph	10.99	11.45	9.81

The starch content is highest at regular harvest, slightly lower at early harvest, and still lower at late harvest periods. Apparently, so far as starch is concerned, the harvest could be started a week earlier, but some loss would result if the harvest were delayed much past the regular period. If the crop weight loss is considered, however, early harvesting becomes as impracticable as late harvesting. Regular harvest has the double advantage of maximum starch content and maximum crop weight.

While it is thought that the type of soil and the previous crop are factors which would have some effect on the starch content of potatoes, the number of samples examined was not sufficient to permit definite conclusions to be drawn. It was observed that at regular harvest and with potatoes as the previous crop, Irish Cobblers contained 15.19 percent starch when grown on upland soil, 15.41 percent on fine sandy loam, and 13.28 percent on loamy sand. Moreover, regular-harvest Irish Cobblers when grown on fine sandy loam contained 14.31 percent starch when the previous crop was oats (followed by sweetclover, which was a failure), 14.64 percent when the previous crop was alfalfa, and 15.41 percent when the previous crop was potatoes. These trends would have to be supported by additional evidence before it would be safe to draw any generalizations.

Varietal differences were very noticeable in the samples of sweetpotatoes analyzed. In all cases (table 9) the Nancy Hall contained the highest percentage of starch and Red Bermuda the least, with Little Stem Jersey and Big Stem Jersey intermediate. In this connection, it is worthy of note that the Mississippi Agricultural Experi-

ment Station in 1936 (*7, p. 1342*) found the Nancy Hall to contain 22.0 per cent of starches as compared with the 21.5 percent shown in table 9.

TABLE 9.—*Variety comparison with sweetpotatoes from different producing areas in Kansas*

Variety	Starch (fresh-weight basis) in sweetpotatoes from—	
	Kansas River Valley	Arkansas River Valley
	Percent	*Percent*
Nancy Hall	21.59	21.49
		19.69
Improved Big Stem Jersey	18.69	
Big Stem Jersey		18.94
Little Stem Jersey	16.88	18.84
	16.57	17.39
Red Bermuda	14.50	

Some variation was observed between the potatoes grown in the Kansas and Arkansas River Valleys, but this also could be attributed to other factors.

A slight increase in starch content was observed in the regular-harvest samples over the early-harvest samples. Also, as would be expected, the curing process brought about a decrease in starch content (table 10).

TABLE 10.—*Effect of harvest period and curing* [1]

Variety	Starch in sweetpotatoes		
	Early harvest	Regular harvest	Cured
	Percent	*Percent*	*Percent*
Nancy Hall	21.06	21.59	18.96
Improved Big Stem Jersey		18.69	17.83
Little Stem Jersey	15.21	16.57	15.33

[1] All samples from Kansas River Valley.

Among the sweetpotato varieties, the Nancy Hall contained the most starch and Red Bermuda the least, with Big Stem Jersey and Little Stem Jersey intermediate.

No important difference was observed between the sweetpotatoes grown in the Kansas River Valley and those grown in the Arkansas River Valley.

The regular-harvest sweetpotatoes had a slightly higher starch content than the early-harvest sweetpotatoes.

Curing of sweetpotatoes caused a decrease in starch content.

SUMMARY AND CONCLUSIONS

Thirty-six samples—18 of potatoes and 18 of sweetpotatoes—were analyzed by the direct acid hydrolysis method and the diastase hydrolysis method of the Association of Official Agricultural Chemists.

Studies on the relative effectiveness of cold storage and shed storage for potatoes showed that the decrease in starch was about the same in both, namely between 4 and 5 percent. Moisture loss of the shed-storage sample amounted to about 35 percent of its original weight as compared with a loss of about 11 percent for the cold-atorage sample.

Varietal differences in potatoes indicated the order of starch content to be Irish Cobbler, Warba, and Bliss Triumph.

The starch content of the late-harvest potatoes was decidedly lower than that of either the regular- or the early-harvest potatoes. No pronounced difference was observed in these last two groups. When the crop weight loss of early-harvest potatoes is considered, it is obvious that a certain stage of maturity must be reached before harvesting begins.

As to soil types, the sandy loam seemed to be slightly better than loamy sand, but the results are not to be regarded as conclusive.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis. Compiled by the committee on editing methods of analysis. Ed. 4, 710 pp., illus. Washington, D. C.

(2) Balch, R. T. and Phillips, J. K.
1941. determination of starch by the a. o. a. c. malt-diastase method. effect of pretreatment of samples. Indus. and Engin. Chem. Analyt. Ed., 13: 815–818.

(3) Barker, J.
1936. analytical studies in plant respiration. vi. the relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. part 3.—the relation of the respiration to the concentration of sucrose. Roy. Soc. London Proc., Ser. B, 119: 453–473, illus.

(4) Cutter, W. P.
1891. test of starch and moisture. Pp. 11–15. *In* Sanborn, J. W., Potato Trials. Utah Agr. Expt. Sta. Bul. 5, 22 pp.

(5) Etheredge, M. P.
1941. a survey of methods for the quantitative estimation of starch. Assoc. Off. Agr. Chem. Jour. 24: 113–119.

(6) Headden, W. P.
1927. effects of nitrates on composition of the potato. Colo. Agr. Expt. Sta. Bul. 325, 96 pp., illus.

(7) Paine, H. S., Thurber, F. H., Balch, R. T., and Richee, W. R.
1938. manufacture of sweet potato starch in the united states. Indus. and Engin. Chem. 30: 1331–1348, illus.

(8) Thurber, F. H.
1933. chemical and physical properties of sweet potato starch. Indus. and Engin. Chem. 25: 565–568, illus.

(9) Watson, T. L.
1895. a chemical study of the irish potato. part i. analyses of the tubers. Va. Agr. Expt. Sta. Bul. 55: (97)–113.

(10) ———
a chemical study of the irish potato. part ii. comparison of the tubers grown in different states. Va. Agr. Expt. Bul. 56: [115]–144.

(11) Widtsoe, J. A.
1897. report of the chemist. i. the composition of potatoes. Utah Agr. Expt. Sta. Ann. Rpt. 8: 22–25.

EFFECT OF STORAGE CONDITIONS ON THE VIABILITY OF TOBACCO SEED[1]

By RANDALL R. KINCAID
Associate plant pathologist, Florida Agricultural Experiment Station

INTRODUCTION AND REVIEW OF LITERATURE

Seed of cigar-wrapper tobacco stored in ordinary rooms in Florida for more than 2 or 3 years seldom remains sufficiently viable to plant. Farmers who sow seed older than this without testing the germination are often disappointed in the resulting stand and vigor of the seedlings. Likewise, in Puerto Rico, "the Tobacco Institute does not recommend the use of tobacco seed after the second year."[2]

On the other hand, tobacco seed has been reported to retain its viability for much longer periods in colder climates. Johnson, Murwin, and Ogden (*4*)[3] reported that in Wisconsin, "seed that was originally of good germinating capacity may be quite satisfactory for commercial purposes when 10 years old." It is reported (*2*) that in Connecticut, "high testing seed in general shows very little diminution in germinative capacity up to 5 years; after that the percentage usually falls gradually, although occasionally there is excellent germination at 10 years."

Shamel and Cobey (*7*) stated that "thoroughly dry seed may be shelled and stored in glass vials or bottles with perfect safety, and can be kept almost indefinitely in this way; the fully matured and dry tobacco seed will retain its viability when kept dry for 10 years, or, as has been observed in several cases, a much longer time."

Chirkovskiĭ (*1*) reported that optimum storage conditions for tobacco seed are low temperature, but above freezing, and low relative humidity.

Poptzoff (*6*) found that the higher the storage temperature used for tobacco seed, the lower must be the relative humidity. He further claims that there is no such thing as a lower limit of relative humidity, for the lower it is, the greater the certainty of conserving the quality of the seed.

EXPERIMENTAL WORK

An experiment to determine some of the factors affecting the longevity of tobacco seed in storage was started at Quincy, Fla. in August 1931 with freshly harvested seed of a cigar-wrapper variety, No. 301. The original moisture content of the seed was estimated by drying to constant weight at 102° C.

Three series of tests were conducted, as follows:

1. Seed was stored in small vials enclosed in 4-ounce screw-cap glass jars over various chemicals, which kept the relative humidity of the air fairly constant at values determined from chemical reference works. The chemicals used were anhydrous calcium chloride ($CaCl_2$), Rochelle salt ($KNaC_4H_4O_6 \cdot 4H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), a

[1] Received for publication February 2, 1943.
[2] Letter from Dr. H. H. Foster, dated June 1, 1942.
[3] Italic numbers in parentheses refer to Literature Cited, p. 410.

saturated solution of ammonium nitrate (NH_4NO_3) with an excess of the salt, and water.

2. Seed was stored in rubber-stoppered vials after the moisture content, originally about 9 percent, had been adjusted to various percentages by placing samples in a moist chamber or a desiccator for various lengths of time. No attempt was made to restore the moisture content to its original value each year when the vials were opened for sampling.

3. Seed with the original moisture content was stored in rubber-stoppered vials, paper envelopes, and cloth bags, and kept in various locations, as follows: Refrigerator, electric, fairly constant at 5° C.; basement room, unheated; laboratory, intermittently heated in winter; and an attic, cold in winter and extremely hot in summer.

No attempt was made to control the amount of light received by the seed during storage. All containers were exposed to diffused daylight at least part of the time.

In October 1932 and at intervals of about a year thereafter, each lot of seed in storage was poured out, mixed, and sampled for the germination test. Any desiccants which appeared to have deteriorated were renewed, and the seeds were returned to storage.

Tests were made on triplicate samples of 100 seeds each, in Petri dishes on two sheets of wet filter paper. The light requirement for the germination of these seeds at constant temperature (*5*) was satisfied by opening the incubator nearly every day. The testing procedure provided favorable conditions for germination, as shown by the fact that every year some tests averaged 80 percent or more. At convenient intervals of a few days, seedlings were counted and removed from the dishes, until further incubation of a few days gave little or no further germination. The averages of triplicate germination tests for each of the 11 years of the experiment to date are given in table 1.

Seeds stored in the refrigerator over the three salts, calcium chloride, Rochelle salt, and ferrous sulfate, retained their viability for 11 years. Those stored over a saturated solution of ammonium nitrate deteriorated considerably, and those stored over water were all dead at the end of 1 year.

Seeds stored in either the basement, laboratory, or attic over calcium chloride retained their viability for 11 years. Those stored over the other salts or the salt solution were all dead after from 2 to 5 years, and those stored over water were all dead at the end of 1 year

Seeds stored in stoppered vials with an original moisture content of 5.3 percent or less showed a small percentage of germination after 8 years; they would probably have remained viable much longer if the original moisture content had been restored at suitable intervals. With each increment in original moisture content the survival period decreased, until at 10.7 percent moisture, nearly all the seeds were dead after 1 year.

Seeds stored in stoppered vials, paper envelopes, or cloth bags kept well in the refrigerator, but in the other three locations all or nearly all were dead after 3 years.

Samples from each of the nine lots of seed which germinated 79 percent or more after 10 years in storage were sowed in an ordinary outdoor plant bed in 1942. The stand and growth appeared just as good as were obtained with fresh seed of the same variety.

TABLE 1.—*Germination of tobacco seed after storage under various conditions and for various periods*

How stored	Where stored	Approximate relative humidity inside container	Original moisture content of seed	Germination after number of years indicated										
				1	2	3	4	5	6	7	8	9	10	11
Over chemicals:		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
$CaCl_2$	Refrigerator	1	9	85	50	23	58	76	73	48	78	84	82	88
$KNa(C_4H_4O_6).4H_2O$	do	30	9	86	45	19	60	87	74	67	85	80	85	89
$FeSO_4.7H_2O$	do	[1] 50	9	79	34	23	58	80	70	55	86	86	88	82
NH_4NO_3 [2]	do	70	9	80	70	63	70	73	67	51	58	49	51	42
H_2O	do	100	9	0										
$CaCl_2$	Basement	1	9	65	54	53	69	86	84	81	87	87	91	86
$KNa(C_4H_4O_6).4H_2O$	do	40	9	51	0	0	0							
$FeSO_4.7H_2O$	do	60	9	65	6	0	0							
NH_4NO_3 [2]	do	60	9	81	60	47								
H_2O	do	100	9	0										
$CaCl_2$	Laboratory	1	9	78	52	46	76	57	83	72	75	87	83	79
$KNa(C_4H_4O_6).4H_2O$	do	40	9	77	45	30	1	0						
$FeSO_4.7H_2O$	do	60	9	82	49	40	11	0						
NH_4NO_3 [2]	do	60	9	83	55	60	3	0						
H_2O	do	100	9	0										
$CaCl_2$	Attic	1	9	81	56	60	80	82	79	77	81	74	79	83
$KNa(C_4H_4O_6).4H_2O$	do	40	9	76	54	43	0	0						
$FeSO_4.7H_2O$	do	60	9	67	16	2	0							
NH_4NO_3 [2]	do	60	9	81	31	0	0							
H_2O	do	100	9	0										
In stoppered vials	Laboratory		2.8	89	63	84	77	62	27	14	2	0		
	do		4.3	80	71	79	68	66	36	7	3	0		
	do		5.3	92	78	80	77	69	46	12	11	0	0	
	do		6.8	87	80	68	41	18	3	0				
	do		8.0	79	64	57	16	0						
	do		9.0	70	25	0	0							
	do		10.7	2	0	0	0							
In various containers:														
Stoppered vial	Refrigerator		9	76	49	24	60	89	75	65	81	83	86	83
Paper envelop	do		9	77		14	35	85	68	69	81	82	85	84
Cloth bag	do		9	61		20	70	78	77	70	77	83	81	73
Stoppered vial	Basement		9	63	8	0	0							
Stoppered vial	Laboratory		9	60	16	0	0							
Paper envelop	do		9	63	28	1	0							
Cloth bag	do		9	72	29	2	0							
Stoppered vial	Attic		9	49	1	0	0							

[1] Estimated from values found for $CoSO_4.7H_2O$.
[2] Saturated solution.

It is planned to continue the experiment at least as long as any of the samples keep satisfactory viability.

DISCUSSION

Practical methods for storing tobacco seed for 11 years, perhaps much longer, are suggested by the results of this experiment.

Ordinary desiccators containing calcium chloride, or probably any other good desiccant, may be used. A desiccator can be improvised from any tight container of glass or metal, using anhydrous calcium chloride and a false bottom to support the seed. Drying the seed in a desiccator and then storing it in a tight container would probably be satisfactory.

Storage in an electric refrigerator at a temperature a few degrees above freezing, with the seed enclosed in ordinary containers, gave as good results as dry storage at any temperature tested. Storage in an ice refrigerator would probably not be as satisfactory, because of the higher humidity and usually higher temperature.

Farmers who produce a supply of tobacco seed sufficient for several years may preserve it for many years by suitable storage. Such a

supply of seed may be valued more highly after one or more satisfactory crops have been produced from it. The importance of being able to preserve experimental lots of tobacco seed for long periods need not be discussed here.

Several samples, especially those stored in the refrigerator, showed a decline in germination test during the first 3 years in storage, and afterwards returned to a high test. Similar results have been reported by Goodspeed (*3*) and by Johnson, Murwin, and Ogden (*4*). No explanation can be offered, but it seems improbable that this kind of result was due to the conditions of the germination tests, because every year some of the tests averaged 80 percent or more. Another storage experiment has been started, in which this phenomenon will be given special attention.

SUMMARY

Cigar-wrapper tobacco seed of the 1931 crop was placed in storage at Quincy, Fla., immediately after harvest (1) in closed containers over various chemicals, (2) in rubber-stoppered vials with the moisture content of the seed adjusted and determined, and (3) in various containers. Test lots were kept in different locations, as follows: Refrigerator at about 5° C., basement, laboratory, and attic.

After 11 years, seed stored over calcium chloride in each location, and seed stored in various ways in the refrigerator, germinated well in the laboratory. Samples which tested 79 percent or more after 10 years also germinated well in an ordinary outdoor plant bed.

Seed stored in rubber-stoppered vials with an original moisture content of 5.3 percent or less showed a small percentage of germination after 8 years.

Samples stored in ordinary containers in the laboratory were all or nearly all dead after 3 years.

Practical applications of dry or cold storage to commercial and experimental lots of tobacco seed are suggested.

LITERATURE CITED

(1) Chirkovskiĭ, V. I.
1938. The viability of tobacco seed. Tabak 8: 22–24. Chim. & Indus. [Paris] 42: 562. [Abstract in Chem. Abs. 34: 3317. 1940.]

(2) Connecticut Agricultural Experiment Station.
1936. Report of the director for the year ending October 31, 1935. Conn. Agr. Expt. Sta. Bul. 381: 165–202.

(3) Goodspeed, T. H.
1913. Notes on the germination of tobacco seed. Calif. Univ. Pubs. Bot. 5: 199–222.

(4) Johnson, J., Murwin, H. F., and Ogden, W. B.
1930. The germination of tobacco seed. Wisc. Agr. Expt. Sta. Res. Bul. 104. 15 pp., illus.

(5) Kincaid, R. R.
1935. Effects of certain environmental factors on the germination of Florida cigar-wrapper tobacco seeds. Fla. Agr. Expt. Sta. Bul. 277, 47 pp., illus.

(6) Poptzoff, A.
1933. Studies in storage of tobacco seed. Pt. 2. The influence of air humidity, temperature, and air-tight keeping on tobacco seeds during their storage. U. S. S. R., Krasnodar Inst. Tobacco Indus. Bul. 93: 1–58. [In Russian. English summary, pp. 56–58.]

(7) Shamel, A. D., and Cobey, W. W.
1907. Tobacco breeding. U. S. Dept. Agr. Bur. Plant Indus. Bul. 96, 71 pp., illus.

THE EXCRETION OF ARSENIC BY THE MALPIGHIAN TUBES OF GALLERIA MELLONELLA, TENEBRIO MOLITOR, AND RHODOPHORA FLORIDA[1]

By ROBERT L. PATTON

Instructor in insect physiology, Department of Entomology, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

The work herein presented formed part of a program of research dealing with the physiological factors in insect control with insecticidal materials. The ultimate goal of this program is the determination and estimation of basic physiological factors and the improvement of control practice according to the factors involved. As a starting point the elimination of arsenious ion by the organs of excretion was investigated. The rates of absorption of a solution of sodium arsenite of lethal concentration in normal saline were determined and the effects of the arsenious ion upon the activity of certain enzyme systems were assayed.

The specific purpose of the investigation was to estimate the part played by the excretory system of insects of different degrees of susceptibility in the elimination of arsenic.

PREVIOUS WORK

Several attempts have been made to determine how arsenic acts on insects and why some insects are resistant to it. Fink (*3*)[2] measured the respiratory rates and the respiratory quotients of potato beetles and wireworms which had been fed various arsenicals, and found that in general the oxygen intake was reduced and the respiratory quotient was increased. Following this study Fink (*4*) determined the effects of arsenic upon the glutathione content of a series of insects. He found the content reduced with arsenic poisoning, but observed that in *Malacosoma americana*, his most resistant species, the initial glutathione content was the lowest. In a third series of investigations, Fink (*5*) studied the activities of arsenic upon amylase, lactase, maltase, invertase, lipase, and protease from potato beetles. No significant effects were observed in the presence of paris green and lead arsenate. Parfentjev and Devrient (*9*) observed no significant changes in the respiratory quotient of cockroaches poisoned with arsenic, and the respiration of tissues (muscle, midgut, and nerve tissue) dipped in a solution containing a lethal concentration of arsenic showed no significant reduction. Voskresenskaya (*12*) described the effects of sodium arsenite upon the rate of passage of food through the alimentary canal, its periodic concentration within the tissues of the insect, and the effects of arsenic upon the anterior sphincter of the midgut. She concluded that resistance depends largely upon the

[1] Received for publication November 11, 1942.

[2] Italic numbers in parenthesis refer to Literature Cited, p. 414.

ability of the insect to regurgitate the dose that has been taken in with the food and to maintain a constant relation between the rate of absorption and the rate of elimination of the material from the blood.

MATERIALS AND METHODS

Three species of insects were used in this study, namely, last instar larvae of *Galleria mellonella* L.; late instar larvae of *Tenebrio molitor* L., both of these from laboratory cultures; and last instar larvae of *Rhodophora florida* Gn., a noctuid collected from its host plant, the evening primrose.

The median lethal dose (M. L. D.) of the arsenious ion was determined for *Galleria mellonella* and *Tenebrio molitor* by buccal injection of measured doses. The solution used was prepared from reagent grade (Baker's) arsenious oxide and sodium hydroxide. The pH of the final product was adjusted to neutrality. The sigmoid mortality curve derived from these measurements was rectified by the method of probits described by Bliss (*1*). Unfavorable weather conditions prevented the collection of sufficient numbers of *Rhodophora florida* to make possible the construction of mortality curves.

The rate-of-absorption measurements were made by the method described by Patton and Craig (*10*). In order to eliminate the factor of individual variation, two absorption gages were used simultaneously with each insect, one containing normal saline and a loop of Malpighian tube and the other containing a symmetrical loop from the same larva and a saline made 0.01 N with sodium arsenite.[3] The normal saline was prepared in a stock solution by dissolving 1.95 gm. of NaCl, 1.52 gm. of KCl, and 1.00 gm. of $CaCl_2$ in distilled water and making up to a volume of 100 ml. at 20° C. The pH was adjusted to neutrality by adding sodium bicarbonate or hydrochloric acid. This stock solution was adjusted to an osmotic pressure nearly corresponding to that of the normal blood of the insect under investigation. The osmotic pressure values were determined by the capillary method of Halket (*6*) for the lepidopterous larvae and the values were taken from previous work (Patton and Craig (*10*)) for the mealworm. The osmotic pressure of the saline was adjusted by dilution from a curve prepared by determining the freezing-point depression of several known dilutions of the stock saline.

Data on selective absorption were obtained by measuring the electrical conductivity of the solution in the bulb of the gage at regular intervals over a period of 24 hours. Measurements were made by means of a conductivity bridge.

The recovery of arsenic in the hind-gut was determined by inserting a short section of No. 20 white cotton thread incased in a finely drawn glass tube into the hind-gut through the anal opening of the insect during an absorption measurement. Analysis was subsequently made on the thread by a micro Gutzeit method essentially the same as that of Howe (*8*).

Estimation of the presence of certain of the respiratory enzyme systems (monophenol, polyphenol, and indophenol oxidase, peroxidase, and glutathione), and of the autolytic activity of a protease was

[3] This constitutes a calculated lethal concentration based upon figures published by Yeager and Tauber (13) on the blood volume of *Periplaneta fuliginosa*, and by Fay (*2*) on lethal arsenic content of the blood of *P. americana*.

made by using well-known colorimetric tests such as are enumerated by Hawk and Bergeim (7). The protease activity of the tissues comprising the tubes was estimated from the free amino acid content of an incubated brei by the Danielson modification of the method of Folin (Hawk and Bergeim (7)).

RESULTS

As a result of a series of measurements of the rates of absorption, it was demonstrated that in all species upon which observations were made, arsenic was readily taken up by the Malpighian tubes. In *Galleria mellonella*, the more susceptible species (M. L. D.=9γ (0.009 mg.) As $^{+++}$ per gram live weight), it was possible to show a slight decrease in rate of absorption in the presence of arsenic. When these data were evaluated statistically by the method of individual comparison according to Snedecor (*11*), the *Z* chart test showed that the significance of the reduction in rate was barely within the 5 percent level. (Mean difference=0.042, standard deviation=0.149, and the number of pairs=48.) Significance could not be established between differences found in the rates of absorption of normal and arsenic-bearing saline with either *Tenebrio molitor* or *Rhodophora florida*. (M. L. D. for *T. molitor*=12γ (0.012 mg.) As $^{+++}$ per gram; for *R. florida* the M. L. D. was undetermined but is known to have been less than 15γ (0.015 mg.) As $^{+++}$ per gram.)

The qualitative survey of the effects of arsenic upon respiratory enzyme activity showed no appreciable change in the activity of the enzymes demonstrated to be present in the normal Malpighian tissues. No monophenol oxidase, polyphenol oxidase, or glutathione could be demonstrated in any case. The presence of arsenic appeared to have no effect upon the activity of indophenol oxidase, which gave a weak test with Nadi [4] reagent in both treated and untreated samples, or upon the activity of the peroxidase present.

A comparison of the relative concentrations of free amino acid in salines containing nearly equivalent amounts of Malpighian tissue was made after an incubation period of 3 hours. The tubes of *Galleria mellonella* and *Tenebrio molitor* showed nearly equal concentrations of free amino acids in both samples. Apparently protein autolysis is not significantly inhibited by the presence of As $^{+++}$.

That the absorption of arsenic by the tubes is not selective in *Galleria mellonella* and *Tenebrio molitor* was demonstrated by the fact that the electrical conductivity of the saline bathing the tubes was unchanged over a period of 24 hours. During this time an average total volume of 4.9λ (cubic millimeters) of solution was absorbed.

Analysis of the threads which had been inserted into the hind-gut of the larvae of *Galleria mellonella* showed an average recovery of 38 percent of the (calculated) arsenic absorbed by the tubes. Difficulty in collecting the samples made quantitative recovery impossible. Anatomical differences precluded comparable measurements with *Tenebrio molitor*.

DISCUSSION

The results of the foregoing experiments demonstrate that the Malpighian tubes of all three species of insects used in this study

[4] Equal quantities of 1 percent alpha naphthol (in 95 percent alcohol) and 1 percent paraphenylenediamine (in water).

were able to filter the body fluid of the insect and remove soluble salts by a process comparable to the action of vertebrate glomeruli. They also demonstrate that the presence of arsenious ion in a concentration which approximates the lethal concentration has no significant effect upon the ability of the tubes to function in the process of filtration. A lethal dose of arsenic administered buccally to a series of mealworms which were subsequently dissected and the rates of absorption of normal saline determined caused no significant change of rate.

From these data it is apparent that the Malpighian tubes play a definite role in the removal of arsenic from the blood stream of insects poisoned by the ingestion of arsenic-bearing insecticides. There is no significant relation between the susceptibility of the insect and the effects of arsenic upon the tubes.

SUMMARY

The rates of absorption of arsenious ion by the Malpighian tubes of three species of insects—*Galleria mellonella*, *Tenebrio molitor*, and *Rhodophora florida*—were determined and compared. *G. mellonella*, the species most susceptible to arsenic poisoning, showed a reduction in the rate of absorption in the presence of arsenic but the reduction was of indeterminate significance. The other two species showed no significant difference. None of the enzyme systems estimated were affected.

From the measurements made, it is apparent that the Malpighian system of insects plays a definite role in the elimination of arsenic from the blood; however, the data give no indication that the function of these organs is the important factor in the relative susceptibility of insects to arsenic poisoning.

LITERATURE CITED

(1) Bliss, C. I.
1934. The method of probits. Science 79: 38–39, illus.
(2) Fay, R. W.
1942. Distribution of arsenic in the body of the American roach. Jour. Econ. Ent. 35: 45–47.
(3) Fink, D. E.
1926. Physiological studies of the effect of arsenicals on the respiratory metabolism of insects. Jour. Agr. Res. 33: 993–1007, illus.
(4) ———
1927. Is glutathione the arsenic receptor in insects? Jour. Econ. Ent. 20: 794–801, illus.
(5) ———
1932. The digestive enzymes of the Colorado potato beetle and the influence of arsenicals on their activity. Jour. Agr. Res. 45: 471–482.
(6) Halket, A. C.
1913. On various methods for determining osmotic pressures . . . New Phytol. 12: 164–176, illus.
(7) Hawk, P. B., and Bergeim, O.
1937. Practical physiological chemistry. Ed. 11, 968 pp., illus. Philadelphia.
(8) Howe, A. E.
1938. Microdetermination of arsenic. Indus. and Engin. Chem., Analyt. Ed. 10: 226–232, illus.
(9) Parfentjev, J. A., and Devrient, W.
1930. Über die wirkung des arsens auf den gasstoffwechsel bei insekten. Biochem. Ztschr. 217: 368–377.

(10) Patton, R. L., and Craig, R.
1939. The rates of excretion of certain substances by the larvae of the mealworm, Tenebrio molitor L. Jour. Expt. Zool. 81: 437–457, illus.
(11) Snedecor, G. W.
1937. Statistical methods applied to experiments in agriculture and biology. 341 pp., illus. Ames, Iowa.
(12) Voskresenskaya, A. K.
1939. On the principles of resistance of some insects to arsenic insecticides. Bul. Plant Protection (U. S. S. R.) 19: 132–144 illus. [In Russian. English summary p. 144.]
(13) Yeager, J. F., and Tauber, O. E.
1932. Determination of total blood volume in the cockroach, P. fuliginosa, with special reference to method. Ent. Soc. Amer. Ann. 25: 315–327.

U. S. GOVERNMENT PRINTING OFFICE: 1943